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The study of behavioral dysfunctions

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2000

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

van der Staay, F. J. (2000). *The study of behavioral dysfunctions: an evaluation of selected animal models*. s.n.

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Behavioral consequences of lesioning the nucleus basalis magnocellularis (nbm) in rats

Census projections indicate a steady increase in the number of aged people (Martin, 1991; Holden, 1996; Butler, 1997) because life expectancy is increasing by 2 years each decade (Raleigh, 1997). Concomitantly with an increase of the proportion of the aged in the population (Butler, 1997), the number of patients suffering from Alzheimer's disease will increase dramatically (e.g. Brody, 1985; Anderson, 1986; Butler, 1997; Molnar & Dalziel, 1997). About half of the people aged 85 years suffer from Alzheimer's disease, and it is exactly this age group that is growing faster than any other age group (Marx, 1996). Dementia of the Alzheimer type (DAT), a progressive clinical state characterized by a deterioration of intellect, memory, judgment, and abstract thinking, and eventually, impaired ability to carry out motor activities despite intact motor functions (American Psychiatric Association: DSM IV, 1994), is among the most disabling impairments of elderly people.

Symptomatology of Alzheimer's disease

One of the earliest, and perhaps the most conspicuous, symptom of the Alzheimer symptomatology is the deterioration of memory functioning (Flicker et al., 1985; Morris & Kopelman, 1986), the amnesic syndrome (Stam, 1987). This syndrome, which is considered as a first-order symptom of DAT (Stam, 1987), consists of a complex of symptoms: memory deficits for recent events and disorientation in time, space, and person. Memory dysfunction first manifests itself as an increase in forgetfulness. However, in the early stage of the disease it is often difficult to distinguish between forgetfulness as early sign of DAT and normal, age-associated memory impairment (AAMI). Patients appear to be unable to remember facts and events which happened recently in their close environment, they forget what they had just discussed, and may ask the same questions over and over again. This impairment of recent memory appears also to underlie the disorientation seen in Alzheimer's patients. Spatial disorientation first becomes obvious in new or less familiar environments.

It is often claimed that remote memory is spared in patients with Alzheimer's disease. Patients appear to remember events in their youth whereas they are unable to retain what has happened only a few minutes ago. However, the accuracy of remote memory often cannot be verified. In later stages of the disease, remote and long-term memory are definitely impaired (Stam, 1987). As the disease progresses, disorientation worsens as a consequence of impairments of remote memory. The patient now loses the ability to find his or her way in his or her old, familiar environment. Knowledge about the familiar environment is stored in long-term memory, which contains consolidated memory, i.e. information that is available and retrievable over long periods of time (hours to decades) (Stam, 1987).

In later stages of the disease, people suffering from DAT lose their memory of their own life history and knowledge acquired through education and experience. They are suffering from apraxia, i.e. loss "(...) of highly complex and previously learned skills and gestures" (Adams, Victor & Ropper, 1997, p. 56), aphasia, "(...) a loss of production and/or the comprehension of spoken and written language (...)"

(Adams, Victor & Ropper, 1997, p. 477) and agnosia, the inability to perceive or distinguish sensory input which is not caused by pathological changes in the senses (Stam, 1987).

With advancing dementia, judgment, i.e. logical and social abilities, deteriorates drastically. The demented patient shows, for example, deviant social behavior, i.e. behavior that is inappropriate in a particular social context. As the disease progresses, the patient becomes unable to discriminate between important details and minor matters, and to deduce underlying general principles and rules.

Second-order symptoms of DAT are changes in mood and personality (Stam, 1987). Emotional responsiveness undergoes progressive changes. The patient appears to become emotionally unresponsive. Gestures, voice, and face become more and more expressionless. However, at times the patient may be over-reactive and hypersensitive. In advanced stages of Alzheimer's disease, the patient is typically apathic and withdrawn, showing no signs of emotional responsiveness. These symptoms, however, are not specific for DAT. Depressive phases in Alzheimer's patients, for example, might mistakenly be diagnosed as depression, whereas the poor performance of depressive, aged patients in cognitive tasks might mistakenly be diagnosed as symptoms of Alzheimer's dementia.

Stages in Alzheimer's disease

There are three stages in the development of DAT: mild, moderate, and severe (Heinitz, 1997; Molnar & Dalziel, 1997):

In patients with *mild* dementia, personal activities of daily life (PADL) and social activities are impaired, but the patient is able to maintain an acceptable standard of personal hygiene, and judgment is still intact. The patient can live independently in his or her own home under minor supervision and with minor help of a caregiver.

During the *moderate* stage of the disease, the symptoms worsen such that independent functioning of the patient suffering from DAT is only possible if extended supervision and help is provided. The mobility of the patient decreases.

Residential care or long-term institutionalization is often needed during the final, *severe* stage of DAT, in which the disease has developed its full-blown symptomatology. The patient shows severe behavioral dysfunctions and his or her mobility is significantly reduced. He or she is unable to perform PADL without continuous supervision and help from caregivers.

Pathology of Alzheimer's disease

The brains of Alzheimer's patients are characterized by the presence of extracellular senile plaques (SP), intracellular neurofibrillary tangles (NFT) and neuronal and synaptic loss (Giannakopoulos et al., 1997). SP are accumulations of the protein β -amyloid surrounded by dystrophic neurites and glial elements (Greenberg et al., 1996; Giannakopoulos et al., 1997). NFT are composed of abnormal modifications of cytoskeletal components which form paired helical filaments, mainly composed of microtubule-associated, abnormally phosphorylated τ -protein. Macroscopically, pronounced cerebral atrophy is seen (Adams, Victor & Ropper, 1997, p. 1053). None of these alterations, however, are considered as definite biological markers of DAT.

In their large survey of the brains of elderly people with no apparent cognitive deficits, of elderly people with AAMI, and of DAT patients, Giannakopoulos and coworkers (1997) demonstrated that SP and NFT formation are age-related phenomena. However, the distribution of SP and NFT was different among the three groups. Surprisingly, they did not find evidence for the notion that SP deposits correlate with DAT. Compared with non-demented peers, DAT patients had a very high density of NFT

in the entorhinal cortex. Because of these degenerative processes, the entorhinal cortex loses its connections to the hippocampus, amygdala, and the neocortex, structures which are crucially involved in memory (e.g. Albert & Moss, 1992; Zola-Morgan & Squire, 1992).

Giannakopoulos and colleagues (1997) concluded that early degenerative alterations in the hippocampal formation are correlated with the development of AAMI, whereas cellular degeneration in the neocortex is necessary for the development of DAT. Their results show that the patterns of change in the different cortical and subcortical structures are extremely complex.

Genetics of Alzheimer's disease

Estimates of the prevalence of familial DAT range from about 10% (Wong et al., 1997) to less than 1% (Roses, 1996; Adams, Victor & Ropper, 1997, p. 1049). Recently, molecular genetic research has identified several mutations in two genes, called presenilin 1 (PS-1) and presenilin 2 (PS-2) (Kovacs et al., 1996), in addition to earlier found mutations of the amyloid precursor protein (APP) gene, which all appear to play a crucial part in the induction of familial DAT (Borchelt et al., 1997; Kovacs et al., 1996). Interestingly, these mutated gene products affect APP processing which favors amyloidosis, and lead in familial cases to an earlier onset of the disease (Nitsch, 1996). Typically, onset of familial forms is 20 to 30 years earlier than in the non-hereditary, i.e. sporadic cases. Moreover, susceptibility for both familial and sporadic forms of DAT appears to be associated with the inheritance of the apolipoprotein (APO) E4 allele (Roses, 1996).

The familial and sporadic forms of DAT appear to be phenotypically different. Based on the DAT-related genes and gene mutations identified thus far, Roses (1996) distinguished between four types of the disease. The first one is familial and is caused by an autosomal dominant mutation of the APP gene. The second type is late-onset, familial or sporadic and is associated with the susceptibility gene, APO E4. The third type is an early-onset familial form and is associated with mutation(s) of the autosomal dominant PS-1 gene. The fourth type is familial and is related to mutations(s) of the autosomal dominant PS-2 gene.

Although only a very small proportion of all DAT cases appears to be inherited, studies of DAT expect that understanding of the hereditary forms will significantly contribute to our understanding of non-hereditary forms. Therefore, efforts are being made to reproduce the DAT pathology in rodents through the introduction of human transgenes carrying the disease-causing gene mutation(s) (e.g. Loring et al., 1996). While keeping in mind the heterogeneous phenotypes of different forms of DAT, the hereditary forms are believed to provide appropriate models of DAT. Attempts to design transgenic animal models expressing the key pathological changes of Alzheimer's disease, SP and NFT, have been made for nearly one decade. Unfortunately, this line of research has already suffered major drawbacks (Marx, 1992; Greenberg et al., 1996). Although a key pathological feature, i.e. the massive deposit of SP has been modeled in transgenic mice, no functional deficits have been detected. It remains to be seen whether additional key pathological features can be modeled which will cause the behavioral dysfunctions seen in Alzheimer's disease. It is unlikely that therapies for the key pathological changes of DAT will become available in the near future. Despite an intense search for drugs to treat Alzheimer's disease, the therapeutic state is still low.

Changes in neurotransmitter systems associated with Alzheimer's dementia

During the last two decades, approaches to find putative Alzheimer's therapeutics have focused on the deterioration of neurotransmitter systems seen in DAT (e.g. Robbins et al., 1997). These therapeutics are expected to preserve or improve cognitive functioning (Jaen & Davis, 1993; Molnar & Dalziel,

1997), especially memory performance, and to slow down or to halt the progressive deterioration seen in Alzheimer's patients (Marx, 1996), thereby delaying the time when the patient becomes dependent on others to manage his or her PADL.

The activity of many neurotransmitter systems is decreased in demented patients (Edwardson et al., 1986; Whalley, 1989; Bierer et al., 1995), but the strongest decrease appears to occur in the cholinergic system (Jacobs & Butcher, 1986; Fibiger, 1991; Bierer et al., 1995). Many different strategies have been proposed for the treatment of this age-related decline of cognitive functions observed in the aged and, more severely, in demented people. However, because pathological changes in cholinergic neurotransmission in Alzheimer's disease appear to be of great importance, a major approach in the development of putative Alzheimer therapeutics focused on the compensation of cholinergic dysfunctions (Dunnett & Barth, 1991; van der Staay, Hinz & Schmidt, 1996a,b).

Neuroanatomically, there is a profound degeneration of acetylcholine (ACh)-releasing cells in the nucleus basalis of Meynert (nbM), localized in the basal forebrain, in patients with DAT (Coyle, Price & DeLong, 1983; Jacobs & Butcher, 1986; Davison, 1987; Vogels et al., 1990). Moreover, there is a very profound increase in NFT in this nucleus in very old patients with DAT (Giannakopoulos et al., 1997), which compromises neuronal functioning. The nbM provides the major cholinergic input to the neocortex.

Cell loss in the nbM and decrease of cholinergic activity in the cortex have also been observed in patients suffering from other types of dementias such as Pick's disease (Uhl et al., 1983; but not confirmed by Tagliavini & Pilleri, 1983), Parkinson's disease (Candy et al., 1983; Whitehouse et al., 1983; Tagliavini et al., 1984), Creutzfeldt-Jakob disease (Arendt, Bigl & Arendt, 1984), and Korsakoff's disease (Arendt et al., 1983). Taken together, these findings provide ample evidence that the degeneration and loss of cholinergic cells provide a morphological correlate of the reduced cortical cholinergic activity that might play a crucial role in several types of dementias. The precise relation between cell loss and reduced activity of cortical choline acetyltransferase (ChAT), however, remains to be determined (Plotkin & Jarvik, 1986).

The degree of cholinergic dysfunction seems to be correlated with the severity of the dementia (Bierer et al., 1995). Moreover, a clear correlation between the severity of the cognitive impairments and the severity of the histological abnormalities (senile plaques – but see Giannakopoulos et al., 1997 – and the reduction of cortical ChAT activity) has been found in patients with Alzheimer's disease (e.g. Perry et al., 1978; Wilcock et al., 1982; Davies, 1985; Katzman, 1986; Whalley, 1989). There is also evidence that the dysfunction of cholinergic systems contributes to the neuropsychiatric changes, particularly psychosis, agitation, anxiety, and depression, seen in patients suffering from Alzheimer's disease (Cummings & Kaufer, 1996).

The nucleus basalis-lesioned rodent as a model of DAT

The nucleus basalis magnocellularis (nbm) is considered the animal analogue of the nbM in humans (e.g. Wenk, Cribbs & McCall, 1984; Shaughnessy et al., 1994, 1996). The cell bodies of the nbm in rodents appear to be spread rather diffusely in the basal forebrain (Wenk, Cribbs & McCall, 1984). Approximately 80% to 90% of the efferents from the nbm to the neocortex, are cholinergic in rodents (Rye et al., 1984; Smith, 1988; Baskerville, Chang & Herron, 1993). The cholinergic projections originating in the nbm of the rat are represented schematically in Figure 1. Two additional groups of cholinergic nuclei have been identified in the basal forebrain: the diagonal band of Broca (dbB), and the medial septal area (msa; Wenk, Cribbs & McCall, 1984). The dbB projects predominantly to the

cingulate and the occipital cortices, whereas the msa sends cholinergic projections to the hippocampus. The mammalian cholinergic systems have been systematically reviewed by Woolf (1991).

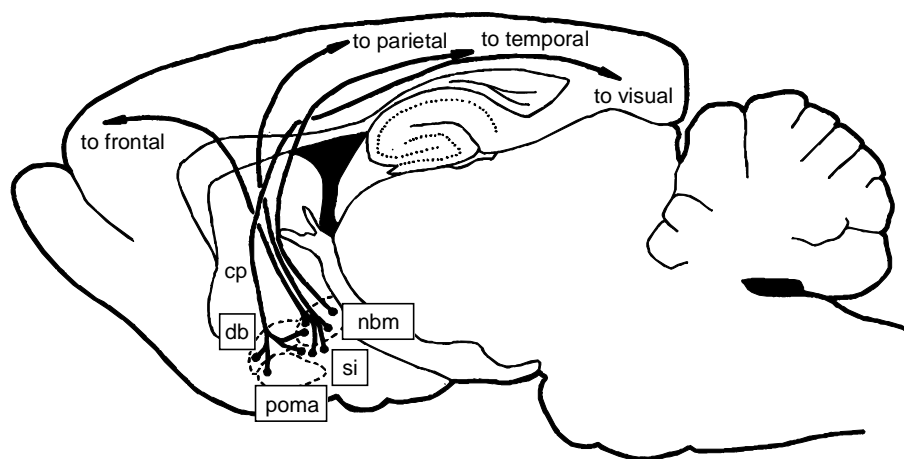


Figure 1. Cholinergic efferents in the neocortex originating from the nucleus basalis magnocellularis in the rat (redrawn from Butcher & Woolf, 1986, and Woolf, 1991). Abbreviations used: **cp**, caudate-putamen complex; **db**, diagonal band; **nbm**, nucleus basalis magnocellularis; **poma**, magnocellular preoptic area; **si**, substantia innominata; **frontal**, frontal cortex; **parietal**, parietal cortex; **temporal**, temporal cortex; **visual**, visual cortex.

A widely used experimental model of the cell loss seen in the nbM of demented patients consists of inducing lesions in the nbm of rodents (Olton & Wenk, 1987). In most studies, the lesions are produced either electrolytically (e.g. Meyer & Coover, 1996; Ogasawara et al., 1996), or by injecting neurotoxins. Most commonly, the neurotoxins are glutamate analogues, such as kainic, ibotenic, quinolinic, or quisqualic acid (Smith, 1988; Wenk, 1996). However, substances such as colchicine, an anti-mitotic agent that appears to bind to tubulin and disrupts axoplasmatic transport (Mundy, Barone & Tilson, 1990; Shaughnessy et al., 1994, 1996, 1998), and ethyl choline aziridinium (AF64A; Nakamura et al, 1992; Männistö et al., 1993), a compound combining structural similarity with choline, i.e. ethylcholine that is recognized by the high affinity choline transporter, and a highly reactive cytotoxic aziridinium ring (Walsh, 1998), have also been used to produce nbm lesions.

Recently, injections of N-methyl-D-aspartate (NMDA, e.g. Luiten et al., 1995; Wenk, Danysz & Mobley, 1995), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA, Hodges et al., 1996), or of the immunotoxin 192 IgG saporin, a monoclonal antibody against nerve growth factor (NGF) receptors that is coupled to saporin and which inactivates ribosomes (Berger-Sweeney et al., 1994; Chiba et al., 1995; Dornan et al., 1996), have been used to produce nbm lesions. All of these lesion techniques produce a reduction in cortical ChAT activity. Whether or not the hippocampal formation is also affected by the lesion depends upon the size of the lesion and the lesion coordinates (Wenk Cribbs & McCall, 1984). The closer the lesions of the basal forebrain nuclei are to the midline, and the more posterior they are, the higher the incidence of effects on ChAT activity in the dorsal hippocampus. In studies aimed at assessing the role of the nbm on cognitive processes, destruction of projections to the hippocampus should be avoided. Hippocampus lesions *per se* have consistently been found to

impair (spatial) learning and memory (e.g. Okaichi & Oshima, 1990; Stubbley-Weatherly, Harding & Wright, 1996).

Whereas lesions of the nbm in rats have consistently been found to impair retention performance in inhibitory or passive avoidance tasks (e.g. Thal, Dokla & Armstrong, 1988; Casamenti et al., 1989; Männistö et al., 1993; Meyer & Coover, 1996; Ogasawara et al., 1996, but see, for example, Winkler et al., 1995), the effects of nbm lesions in other learning tasks appear to be less consistent (Mundy, Barone & Tilson, 1990; Dunnett, Everitt & Robbins, 1991; Torres et al., 1994). It has been suggested that the nbm has a role in both spatial working memory (WM) (e.g. Wozniak et al., 1989) and spatial reference memory (RM) (e.g. Givens & Olton, 1994).

Description of the experiments performed

In the present chapter, we assessed the effects of nbm lesions produced by bilateral injections of the most widely used neurotoxin, ibotenic acid, in two spatial discrimination tasks. Both tasks, a seven-choice discrimination task in a radial alley maze and the spatial holeboard task, have previously been found to be sensitive to the effects of normal aging (see Raaijmakers, et. al., 1990; van der Staay, van Nies & Raaijmakers, 1990, and Appendix 6.2). The seven-choice task might be considered as a win-stay problem that most probably depends upon an intact RM, whereas the holeboard discrimination task simultaneously assesses both spatial WM and RM (van der Staay, Raaijmakers & Blokland, 1990; van der Staay, van Nies & Raaijmakers, 1990; Beldhuis et al., 1992a,b; Markel et al., 1995).

- In Chapter 3.1 we performed experiments to determine whether both, spatial WM and RM are affected by lesions in the nbm, using a spatial holeboard discrimination task. Previously, using a radial arm maze task in which only a subset of arms contained a food reward, Wirsching and colleagues (1989) found impaired WM and RM in rats which had received quinolinic-induced lesions of the nbm. There is experimental evidence that the WM and RM are independent from one another (e.g. van der Staay, van Nies & Raaijmakers, 1990).

It is advantageous to test the effects of nbm lesions on both memory components (WM and RM) in one and the same task (Dekker, Connor & Thal, 1991) because many factors appear to play a role in rat's performance in learning and memory tasks, most of them being associated with the housing conditions and the testing environment (Andrews, 1996). These factors cannot be controlled as strictly if WM and RM are assessed in different testing paradigms.

- In the first and third experiments of Chapter 3.2, we investigated whether bilateral lesioning of the nbm by ibotenic acid disrupts the acquisition of a seven-choice task in the radial alley maze. In the third experiment, we used a second set of lesion coordinates in addition to that used in the previous experiments. In the second experiment of Chapter 3.2, rats received nbm lesions after they had acquired the seven-choice task and then we assessed the effects of the lesions on retention, acquisition of a new problem, and re-acquisition of the originally acquired problem. We wondered whether the lesions disrupt performance in a task that had been acquired before lesioning the nbm.
- It is conceivable that lesioning of the nbm in aged rodents provides a model of Alzheimer's disease that shares more aspects of the Alzheimer symptomatology than lesioning the nbm of young rodents would do. In order to test this hypothesis, we tested young and aged Wistar rats in a battery of behavioral tests, consisting of a seven-choice task in an eight-arm radial alley maze, and a series of sensorimotor tasks. This experiment is described in Chapter 3.3.

3.1

Bilateral lesioning of the nbm in rats: effects on spatial discrimination in the holeboard

Abstract

Postmortem examinations have shown that there is severe cell loss in the nucleus basalis of Meynert (nbM) in patients suffering from Alzheimer's dementia. This cell loss profoundly decreases the number of cortical cholinergic projections and the resulting cholinergic hypofunction has been suggested to be one of the major causes for the cognitive impairments found in Alzheimer's patients. The nucleus basalis magnocellularis (nbm) is considered the rodent analogue of the nbM in humans, and thus lesions of the nbm in animals might mimic the neurodegenerative processes associated with Alzheimer's disease. In the present study we determined whether bilateral lesioning of the nbm in rats affects spatial learning in the holeboard, a task which allows the simultaneous assessment of spatial working memory (WM) and reference memory (RM). Both the WM and RM of the lesioned rats were impaired compared with those of intact or sham-lesioned control rats. This finding supports the notion that the nbm has a role in both WM and RM.

Introduction

The nucleus basalis magnocellularis (nbm) is considered the rodent analogue of the nucleus basalis of Meynert in humans (Smith, 1988). In patients suffering from Alzheimer's disease, a progressive dementia, there is a massive loss of cells in the nbM. Of the cortical projections originating in the nbM, more than 90% are cholinergic in both primates and humans. In rodents, most of the projections from the nbm are to the neocortex, predominantly to the frontal and parietal part (Butcher & Woolf, 1986). Of these projections, about 80% to 90% appear to be cholinergic (Smith, 1988).

Cell loss, either due to neurodegenerative processes in the nbM of Alzheimer patients, or due to experimentally induced lesioning of the nbm, leads to a profound decrease in the cortical cholinergic projections and to a reduction in the activity of cortical choline acetyltransferase (ChAT). This enzyme synthesizes acetylcholine (ACh). Cholinergic hypofunction has been suggested to be one of the major causes for the cognitive impairments found in Alzheimer's patients. One approach to mimic the neurodegenerative processes associated with Alzheimer's disease is to lesion the nbm in animals and to assess the effects of this experimentally induced decrease in cortical cholinergic activity on behavior, predominantly cognitive behavior (Smith, 1988).

It has been suggested that the nbm has a role in both working memory (WM; e.g. Wozniak et al., 1989), and reference memory (RM; e.g. Murray & Fibiger, 1985; Givens & Olton, 1994). The spatial WM and RM can be considered as operational definitions of presumably different memory processes (Olton, Becker & Handelmann, 1979; Frick et al., 1995). Evidence for this notion has been provided by various studies, in which different spatial discrimination tests were used (e.g. van Luijckelaar, van der

Staay & Kerbusch, 1989; van der Staay, van Nies & Raaijmakers, 1990; Frick et al., 1995). It remains, however, unclear whether these two spatial memory components are associated with different neuronal substrates (e.g. Olton, Becker & Handelmann, 1979; Kesner, DiMattia & Crutcher, 1987).

The spatial memory of rodents has been assessed in sequential choice or 'alley' mazes such as the Stone 14-unit maze (Goodrick, 1968, 1975; Michel & Klein, 1978; Ingram, 1985; Goldman et al., 1991) and in 'free-choice'-type mazes, such as the circular platform (Barnes, 1979; Barnes et al., 1990; Steckler et al., 1993; Bardgett, Newcomer & Taylor, 1996), the radial maze (aversively motivated radial water maze: Pitsikas & Algeri, 1992; appetitively, i.e. food-motivated, radial maze: Wallace, Krauter & Campbell, 1980a; Wirsching et al., 1989; Marczyński, Artwohl & Marczyńska, 1994; Arendash, Sanberg & Sengstock, 1995; Levin & Torry, 1996), and the Morris water maze (e.g. Gage, Dunnett & Björklund, 1984; Rapp, Rosenberg & Gallagher, 1987; Aaltonen et al., 1991; Abe, Horiuchi & Yoshimura, 1997). The 'free-choice'-type of mazes appear to be sensitive to the effects of aging and of lesioning of the nbm in rats.

The alley mazes consist of a fixed starting position and one correct route to a fixed goal position, where incorrect alternatives such as visits to blind alleys or going back must be avoided. By contrast, in the 'free choice' spatial discrimination tasks (Crannell, 1942; Lachman & Brown, 1957), food can be found in different places, and the rat is free to visit and revisit these baited places and unbaited alternatives in whatever order it wishes. Once a rat has visited a place and consumed the food pellet, its revisits to the same location remain unreinforced. The most efficient behavior is to visit only baited locations, and to visit them only once. The rat must remember a list of places already visited in order to avoid revisits. This list of visits is held in the WM (Olton & Samuelson, 1976), and the information it contains is relevant only within a specific trial. The RM holds trial-independent information about, for example, the locations where the food reward can be found. WM and RM can be assessed simultaneously in free choice mazes such as the radial maze (van Luijtelaa, van der Staay & Kerbusch, 1989; Shapiro & O'Connor, 1992; Marczyński, Artwohl & Marczyńska, 1994), the cone field (van der Staay, Krechting, Blokland & Raaijmakers, 1990), and the holeboard (van der Staay, van Nies & Raaijmakers, 1990; Beldhuis et al., 1992a,b; Markel et al., 1995).

Because of the presumed role of the nbm in both spatial WM (Wozniak et al., 1989) and spatial RM (Murray & Fibiger, 1985; Givens & Olton, 1994), we chose to use the holeboard as a representative of the 'free choice' mazes to assess the effects of bilateral lesioning of the nbm on spatial orientation performance.

Material and Methods

Animals

Twenty male Wistar (Cpb:WU) rats were supplied by CPB, TNO, Zeist, the Netherlands. The animals were housed in standard Makrolon[®] cages and habituated to a reversed day/night cycle (lights on from 20:00 to 8:00). Then, the rats were weight-matched and semi-randomly assigned to an untreated ($n = 6$; mean body weight in grams \pm SEM: 253 ± 4), a sham-lesioned ($n = 6$; 253 grams ± 4), or an nbm-lesioned group ($n = 8$; 252 grams ± 3). The experimental protocol is summarized in Table 1.

Apparatus

We used a holeboard (70 * 70 * 45 cm; see Fig. 2) constructed according to the descriptions given by Oades (1981a). All walls were made of transparent PVC, the floor consisted of gray polyvinyl chloride (PVC). There were 16 holes (diameter of 3.5 cm) in the floor. The distance between the holes was 10 cm. The bottom of each hole consisted of a flattened cone of perforated aluminum, turned upside down. A cup filled with about twenty 45-mg food pellets (Campden Instruments) was placed under each aluminum bottom. The rat could not reach the pellets in these cups, which were to mask potential odor cues emanating from the reward in the baited holes. Thus, rats were unable to discriminate between baited and unbaited holes by olfactory cues (Willner, Wise & Ellis, 1986; van der Staay, van Nies & Raaijmakers, 1990). The holeboard was situated in a room which was equipped with a radial maze, two tables (one with the control equipment and the micro computer), and two chairs. Posters were hanging on the walls. The room was dimly illuminated by two red fluorescent strip lights.

Table 1. Protocol of the experiment assessing the effects of bilateral lesions of the nucleus basalis magnocellularis (nbm) on the acquisition by young Wistar rats of a spatial holeboard discrimination task. The week numbers (*: after arrival at our laboratory) and the treatments and testing procedures are listed.

Week*	Event
0	Arrival at our laboratory of 20 male CPB:Wu rats Individual housing, reversed day/night cycle (lights on from 20:00 to 08:00)
2	Matching on body weight: assignment to control, sham-, or nbm-lesioned group Stereotaxic operations
3	Start of food deprivation and four adaptation sessions in the holeboard
4-6	Acquisition of holeboard task
9	Removal of the brains, histological verification of lesion, biochemical assays

Surgical procedure

The animals received stereotaxically guided lesions in the nbm for which a stereotaxic instrument (David Kopf) was used. The rats were anesthetized with a combination of Vetalar (i.m.: 50 mg/kg) and Rompun (s.c.: 2.25 mg/kg) (Guldin & Markowitsch, 1982). The bilateral nbm lesions were produced by ibotenic acid (4 µg in 0.4 µl phosphate buffer: pH 7.4) injected over 3.5 minutes, using the coordinates: AP -0.5, L 2.3, and DV 7.6 (with respect to bregma and to the surface of the skull; Paxinos & Watson, 1986). The tip of the Hamilton syringe (28 gauge, needle point type 3) was left in place for 5 minutes, and then the syringe was retracted. In the sham-lesioned rats, the syringe was lowered to DV 6.6, i.e. 1 mm above the lesion site; the other coordinates were as in the nbm-lesioned group (Wenk & Röskaeus, 1988; Wenk, Markowska & Olton, 1989). However, substances were not injected in the sham-lesioned group, in order to avoid mechanical damage to the target area. The untreated rats were left undisturbed until behavioral testing began.

In the first 3 to 4 hours after surgery, the ibotenic-acid treated rats had seizures, with profound arching of the back. From previous studies we knew that during this period, the animals gnawed continuously,

mostly their own forepaws (O'Connell, Earley & Leonard, 1994). To prevent this automutilation, we fitted the animals with a ruff which they wore for the first 8 hours after surgery (see Fig. 1).

One week after surgery, all animals except one nbm-lesioned rat had regained or exceeded their pre-operation weights. The nbm-lesioned rat that did not recover well was excluded from the experiment. As matching had been performed on the pre-operation body weights, we evaluated whether the loss of one animal produced unequal groups. The pre-operation body weight of the nbm-lesioned group with only 7 animals was 256 ± 8 grams (mean \pm SEM). Analysis of variance confirmed that the groups still were similar with respect to body weight ($F_{2,16} < 1.0$, n.s.).

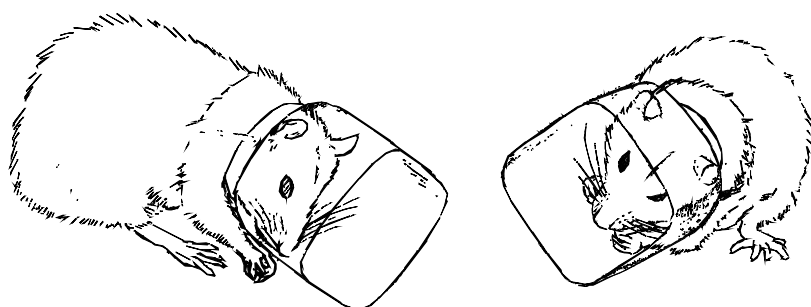


Figure 1. Typical position of ibotenic-acid treated rats in the first hours after surgery. The ruff prevented the rats from hurting themselves.

Behavioral testing in the holeboard

Adaptation sessions: in the second week after surgery, the body weights of all animals were gradually reduced to 85% of their free-feeding values. When the rats had reached their target weights, growth correction was applied during testing, using the growth curves of undeprived male Wistar peers.

Beginning one day after the start of the food-deprivation regimen, the rats were habituated to the holeboard during 10-min adaptation sessions on 4 consecutive days. During adaptation all holes were baited with one 45-mg food pellet. Additional pellets were scattered randomly on the floor of the holeboard during the first 2 days.

Acquisition of holeboard discrimination: the rats were then trained to collect pellets from a fixed set of four holes. A rat was placed in a clear Plexiglas start-box which could be connected to the holeboard in the middle of one side wall. A trial was initiated by raising the guillotine door between the start-box and the holeboard; it was terminated when the rat found all the food pellets or when 10 minutes had elapsed, whichever event occurred first. Hole visits were registered manually, using a keyboard with 16 keys (representing the 16 holes) connected to an Apple //e microcomputer. A hole visit was scored when a rat turned its nose to the edge of a hole, moved its nose over the hole, or poked its nose in the hole (Oades, 1981a). In addition, infrared photocells detected automatically whether a rat poked its nose into a hole. The configuration of baited holes is depicted in Fig. 2. The rats were trained with massed trials on 16 consecutive days (days 1 and 2: 2 trials/day, days 3 to 16: 4 trials/day) to a total of 60 trials. All testing was done between 9:00 and 13:00.

After completion of the sixtieth trial, rats were returned to an ad libitum feeding regimen for 3 weeks. Then they were decapitated, the frontal cortex was dissected free, and the choline acetyltransferase activity of this brain sample was determined.

The mean body weights (\pm SEM), three weeks after the end of behavioral testing were 372 grams (\pm 17) for the untreated, 364 grams (\pm 20) for the sham-lesioned rats, and 359 grams (\pm 23) for the nbm-lesioned rats. Analysis of variance (ANOVA) confirmed that all groups had recovered similarly ($F_{2,16} < 1.0$, n.s.)

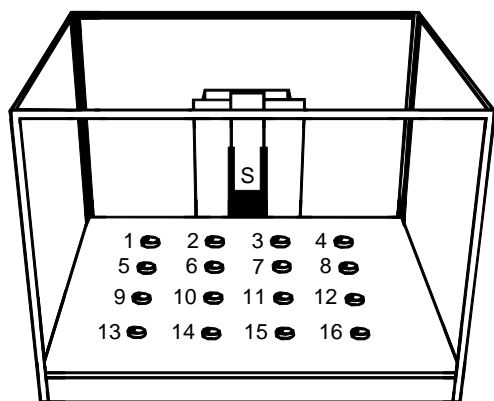


Figure 2. The holeboard apparatus is an open-field with 16 holes in the floor. The startbox (S) is connected to the holeboard; opening a guillotine door gives access to the holeboard. The holes no. 4, 6, 11, and 13 were baited with one 45-mg food pellet each.

Statistical analysis

Adaptation sessions: one measure of the adaptation sessions was analyzed statistically:

- Number of nose-pokes, i.e. the number of times a rat poked its nose into a hole, deep enough to allow automatic detection by the infrared photocells in the holes. Nose-pokes in a holeboard are generally considered a measure of exploration (e.g. File & Fluck, 1994)

Treatment effects on the adaptation sessions were analyzed by a one factorial ANOVA in which the three lesion conditions (untreated, sham-lesioned, and nbm-lesioned) were considered as three levels of the factor Treatment. Changes in the course of the adaptation period were assessed by a Session (sessions 1 to 4) by Treatment ANOVA with repeated measures on the first factor.

Acquisition of holeboard discrimination

Four measures of formal training were analyzed statistically: WM, RM, mean inter-visit interval, and choice correspondence of reinforced visits.

- WM was defined by the ratio: (number of food rewarded visits)/(number of visits and revisits to the baited set of holes). Thus, this measure represents the percentage of all visits to the baited set of holes that were reinforced with food (van der Staay, Raaijmakers & Collijn, 1986; van der Staay, van Nies & Raaijmakers, 1990).
- RM was defined by the ratio: (number of visits and revisits to the baited set of holes)/(number of visits and revisits to all holes). This measure expresses the number of visits to the baited set of holes as a percentage of the total number of visits to all the holes. As Olton and co-workers (Olton, Becker, Handelsmann, 1979; Olton & Papas, 1979) point out, and as has been confirmed by van der Staay, van Nies, and Raaijmakers (1990) with the holeboard task, the two measures of spatial memory can be considered to be independent of each other because visits to the unbaited holes are not considered in the WM measure.

- The mean inter-visit interval was determined by dividing the time elapsed between the first and last visits in a trial by (number of visits - 1). This variable provides a measure of the speed of visiting the holes.
- Choice correspondence of reinforced visits: this measure compares the sequences of the reinforced hole-visits of two subsequent trials. The longest common sequence was taken as the measure of correspondence. This measure could range from 1 to 4: a score of 1 was awarded when sequences were completely different, and a 4 was scored whenever the sequences of all four reinforced visits were identical (van der Staay, van Nies & Raaijmakers, 1990). This measure reflects the variability in the spatial pattern for collection of the rewards, but it neglects all erroneous choices, i.e. visits to the never baited holes and revisits to holes of the baited set. Computer simulation revealed that the mean choice correspondence is 1.72 if the order of reinforced visits was purely random from trial to trial (see also Appendix 6.1).

Mean block scores of ten trials each were calculated for WM and RM performance, for mean inter-visit interval, and for the choice correspondence of reinforced visits. Effects of lesioning of the nbm were evaluated by a Treatment by Trial blocks ANOVA, with repeated measures on the last factor. In addition, changes in the course of training were evaluated by a one-factorial (Treatment) ANOVA on the scores averaged over all trial blocks (General mean) and on the orthogonal trend components calculated over successive trial blocks. In this ANOVA the three lesion conditions (intact, sham-lesioned, nbm-lesioned) were considered as three levels of the factor Treatment.

The general mean evaluated whether there was a difference in the overall level of performance. Orthogonal trend coefficients were used to describe the learning curves and to assess whether the shapes of these curves were different between groups. These analyses were supplemented by ANOVAs on individual trial blocks. If a particular measure showed a parallel increase or decrease over sessions between treatment conditions, the result of the analyses on general means are reported. If measures diverged or converged between groups in the course of training, then ANOVAs on individual block means are also reported to indicate in which phase of training differences between treatment conditions appeared or disappeared. All analyses were supplemented by planned orthogonal comparisons which contrasted:

- the untreated group with the sham-lesioned group (Contrast I), and
- the untreated and sham-lesioned groups pooled with the nbm-lesioned group (Contrast II).

Fisher's LSD post hoc comparisons are reported when Contrast I identified differences between the intact and the sham-lesioned groups.

Choline acetyltransferase (ChAT) activity in the frontal cortex

The rats tested in the present holeboard experiment and the rats tested in a seven-choice task in a radial alley maze (see Chapter 3.2, first experiment) had been lesioned at the same coordinates. They were of a comparable age and were decapitated after a similar survival period after lesioning. Therefore, the data of the intact, sham-lesioned, and nbm-lesioned animals from both experiments were pooled and analyzed.

Dissection of brain sample: brain samples were dissected eight weeks after lesioning. The rats were decapitated without anesthesia, and the severed head was kept in liquid nitrogen for about 5 seconds to cool the brain. Then, the brain was rapidly removed and dissected at 4-10°C in an open refrigerator .

In the coronal plane, the frontal cortical sample was delimited with a calibrated plastic T-square (Rosenzweig, Bennett & Diamond, 1972; Raaijmakers 1978; see also Chapter 3.2, Fig. 3). The tissue under the area that was covered by the T-square was not included. Rostrally, the frontal cortex sample was delimited by a horizontal knife-cut above the olfactory bulbi.

Processing of brain tissue for measurement of ChAT activity: the brain sample was weighed on a cooled, pre-weighed aluminum block. Then, it was homogenized in 50 vol (2%, w/v) ice-cold 0.32 M sucrose containing 5 mM Tris-HCl (pH 7.4) (six strokes at 1200 rpm and 100 N force; centrifuge-compatible tube; clearance 0.40 mm) with a teflon-in-glass homogenizer designed and built at the workshop of the Psychological Laboratory, University of Nijmegen, The Netherlands.

The homogenate was centrifuged twice at 4°C for 10 minutes at 1000 x g. The pellet was washed between the centrifugation steps with 1.0 ml buffered sucrose. Then, the combined supernatants were centrifuged at 4°C at 17000 x g for 15 minutes. The pellet was resuspended in 20 vol (5% w/v, with respect to fresh weight) buffered sucrose; this crude mitochondrial suspension is referred to as the synaptosomal suspension P₂.

ChAT activity: ChAT activity was measured according to the radiochemical method of Fonnum (1975). ChAT is the enzyme that catalyzes the formation of acetylcholine from acetyl-CoA and choline: $[^{14}\text{C}]\text{Acetyl-CoA} + \text{Choline} \rightarrow [^{14}\text{C}]\text{Acetylcholine (labeled)} + \text{CoA}$.

Twenty µl of the frozen (-60°C) P₂ suspension was mixed with 30 µl sodium phosphate buffer (50 mM, pH 7.4) and 50 µl of a solution of EDTA (20 mM) and Triton X-100 (0.2% v/v). Aliquots (20 µl) of this mixture were taken in triplicate. The incubation medium contained, in final concentrations: choline chloride, 6 mM (Calbiochem); EDTA, 20 mM (pH 7.4); NaCl, 300 mM; neostigmine, 0.1 mM; and $[^{14}\text{C}]\text{Acetyl-CoA}$ (57.6 mCi/mmol, Amersham) diluted with unlabeled Acetyl-CoA (Boehringer) (final concentration 0.2 mM). All solutions were made in sodium phosphate buffer (50 mM, pH 7.4).

The P₂ mixture (20 µl) was placed in a tube on ice and 50 µl of freshly made incubation medium was added. The tube contents were mixed and incubated for 30 minutes at 37°C. The incubation was stopped by the addition of 10 µl 14 % trichloroacetic acid (TCA) and the tubes were placed on ice for 10 minutes. The content of the tubes was then transferred to a scintillation vial containing 1 ml sodium phosphate buffer (10 mM, pH 7.4); the tubes were rinsed with 4 ml of this buffer. Two milliliters of acetonitrile containing 10 mg tetraphenylboron (Kalignost) and 5 ml toluene scintillator (Packard) were added to the scintillation vials (the toluene scintillator contained 5 g PPO and 0.1 g POPOP per liter toluene), which were gently shaken by hand for 5 seconds. The contents were allowed to separate into two layers for 10 minutes. The aqueous phase containing acetyl-CoA was removed; the toluene phase contained ACh. A liquid scintillation spectrometer (Packard TriCarb) was used to measure the radioactivity. The counting efficiency was 92%, as determined with external standards.

The ChAT activity was calculated as the nanomoles acetylcholine per mg protein formed per hour. Total protein was measured in each sample according to the method of Lowry et al. (1951) with the modifications described by Miller (1959).

Statistical analysis

Effects of lesioning on the ChAT activity in the frontal cortex were analyzed by an ANOVA in which the intact (n = 12), sham-lesioned (n = 12), and nbm-lesioned (n = 15) groups were considered as different levels of the factor Lesion. This analysis was supplemented by planned orthogonal comparisons which contrasted:

- the ChAT activity of the untreated group with that of the sham-lesioned group (Contrast I), and
- the ChAT activity of the untreated and sham-lesioned groups pooled with that of the nbm-lesioned group (Contrast II).

Histological verification of lesions

Care was taken not to damage the brain tissue underlying the dissected sample tissue. This part of the brain was stored in 4% formalin solution for histological verification of the nbm lesion. The size and location of the nbm lesions were assessed in coronal sections (40- μ m thick) cut through the entire lesioned area, using a cryostat microtome. The coronal sections chosen for histological verification roughly correspond to the levels -0.3, -0.8, -1.3, and -1.8 mm from bregma in the stereotaxic atlas of Paxinos and Watson (1986). Slide-mounted tissue sections were stained with cresyl fast violet.

Results

Behavioral testing in the holeboard

Adaptation sessions

The three groups of rats increased their number of nose-pokes from the first to the second and third adaptation sessions, whereas the number of nose-pokes did not change, or even decreased slightly in the fourth session, when compared with the number in the third session (Sessions: $F_{3,48} = 37.65$, $p < 0.01$; see Fig. 3). The change in the number of nose-pokes was not different between groups (Sessions by Treatment interaction: $F_{6,48} = 2.19$, n.s.). During the last adaptation session, the number of nose-pokes was virtually identical for the three groups of rats ($F_{2,16} < 1.0$, n.s.).

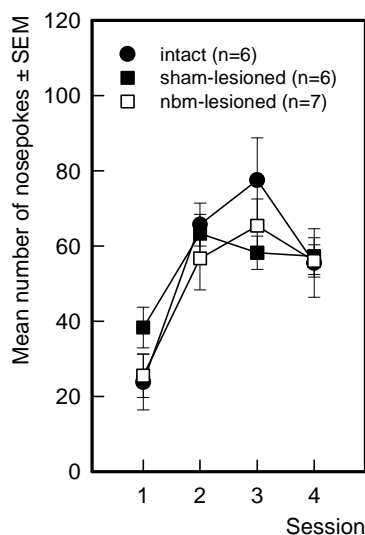


Figure 3. Number of nose-pokes during adaptation sessions of untreated, sham-lesioned and nucleus basalis magnocellularis (nbm)-lesioned male Wistar rats. The session means and the standard errors of the means (SEM) are shown.

Acquisition of holeboard discrimination

WM performance (see Fig. 4, upper left panel): averaged over the six blocks of ten trials, the WM performance was different for the three groups (General mean: $F_{2,16} = 29.2$, $p < 0.01$). The average WM performance of the untreated control group and the sham-lesioned group did not differ (Contrast I: $F_{1,16} < 1.0$, n.s.), whereas the performance of the nbm-lesioned group was impaired (Contrast II: $F_{1,16} = 57.93$, $p < 0.01$).

The WM improved over the trial blocks ($F_{5,80} = 39.33$, $p < 0.01$) to a similar extent in the three groups (Trial blocks by Lesion interaction: $F_{10,80} < 1.0$, n.s.).

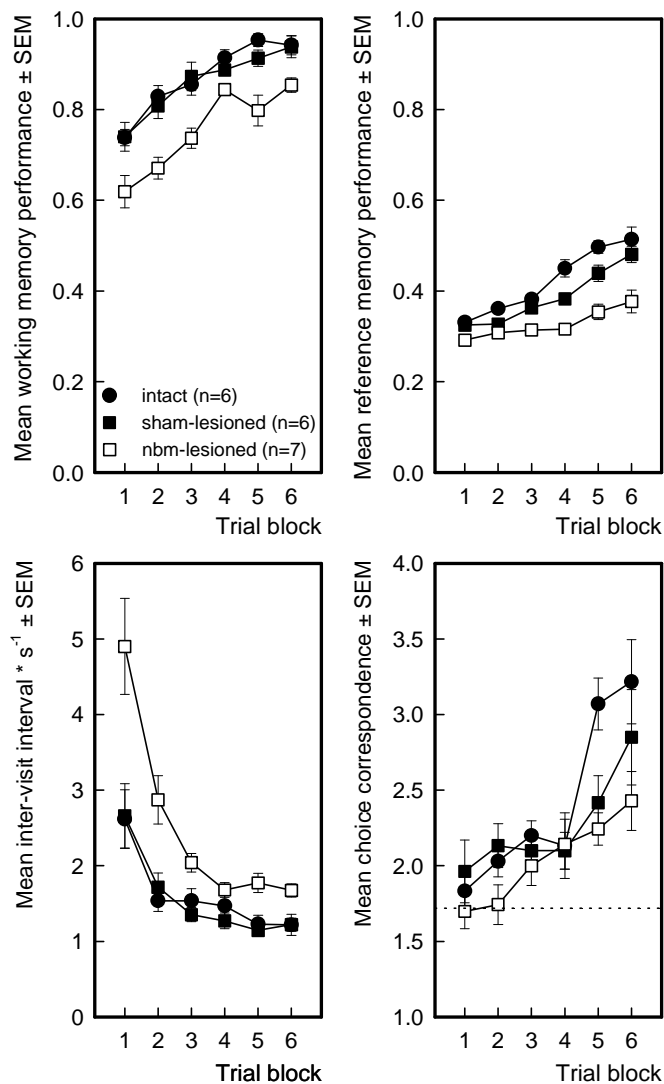


Figure 4. Working memory performance (upper left panel), reference memory performance (upper right panel), inter-visit intervals (lower left panel), and choice correspondence of reinforced visits (lower right panel) of untreated, sham-lesioned, and nucleus basalis magnocellularis (nbm)-lesioned male Wistar rats. The means and the standard errors of the means (SEM) are depicted. The dotted line in the lower right panel indicates the chance level for choice correspondence.

RM performance (see Fig. 4, upper right panel): averaged over the six trial blocks, RM performance was different for the three groups of rats (General mean: $F_{2,16} = 26.75$, $p < 0.01$). The average RM performance of the untreated control group and the sham-lesioned group was different (Contrast I: $F_{1,16} = 6.90$, $p < 0.05$). Fisher's LSD post hoc comparisons of the general means confirmed that all

three groups performed differently; the intact rats performed the best, the nbm-lesioned rats performed the worst. The RM performance improved across the six successive trial blocks ($F_{5,80} = 63.24$, $p < 0.01$). This improvement was different for the three groups of rats (Trial blocks by Lesion interaction: $F_{10,80} < 1.0$, n.s.). The learning curves of the intact rats and of the sham-lesioned rats did not differ from one another (Contrast I: $F_{5,80} = 1.72$, n.s.), whereas the nbm lesion retarded improvement of RM performance (Contrast II: $F_{5,80} = 7.25$, $p < 0.01$).

Inter-visit intervals (see Fig. 4, lower left panel): the average inter-visit interval was different for the three groups (General mean: $F_{2,16} = 9.35$, $p < 0.01$). The intact control rats and the sham-lesioned rats did not differ from one another with respect to the average time needed to visit a hole (Contrast I: $F_{1,16} < 1.0$, n.s.). These two groups pooled needed, on average, less time to visit a hole than the nbm-lesioned rats did (Contrast II: $F_{1,16} = 18.86$, $p < 0.01$); this difference was already apparent in the first trial block ($F_{2,16} = 6.74$, $p < 0.05$).

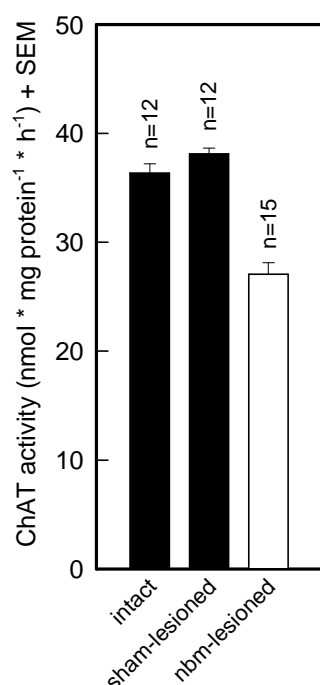


Figure 5. Effect of lesioning the nucleus basalis magnocellularis (nbm) in adult Wistar rats. The mean ChAT activity in the frontal cortex, calculated as nanomoles acetylcholine formed per milligram protein per hour, and the standard errors of the means (SEM) are shown for intact, sham-lesioned, and nbm-lesioned rats.

Note that the rats tested in the present holeboard experiment and the rats tested in a seven-choice task in a radial alley maze (see Chapter 3.2, first experiment) had been lesioned at the same coordinates, were of a comparable age and were decapitated after a similar survival period. Therefore, the data of the intact, sham-lesioned, and nbm-lesioned animals from both experiments were pooled. The sizes of the pooled groups are depicted above the bars.

The inter-visit interval decreased in the course of training (Trial blocks: $F_{5,80} = 46.63$, $p < 0.01$), and this decrease was affected by the treatments (Trial blocks by Lesion interaction: $F_{10,80} = 4.99$, $p < 0.01$). The decrease was not different for the intact and the sham-lesioned rats (Contrast I: $F_{5,80} < 1.0$, $p < 0.01$), but the decrease of the nbm-lesioned rats was different from that of the intact and sham-lesioned rats pooled. (Contrast II: $F_{5,80} = 9.72$, $p < 0.01$). The decrease was predominantly linear (Linear trend component: $F_{1,16} = 60.56$). This trend component covered 71 percent of the variation over trial blocks. The linear component of the decrease in inter-visit interval over trial blocks was similar for the intact and the sham-lesioned rats (Contrast I: $F_{1,16} < 1.0$), whereas there was a steeper linear decrease in the nbm-lesioned rats (Contrast II: $F_{1,16} = 12.03$, $p < 0.01$).

Choice correspondence of reinforced visits (see Fig. 4, lower right panel): the choice correspondence, averaged over the six trial blocks, was different between the groups (General mean: $F_{2,16} = 4.22$, $p < 0.05$). The intact rats and the sham-lesioned rats did not differ for the mean choice correspondence (Contrast I: $F_{1,16} = 1.31$, n.s.), whereas that of the nbm-lesioned rats was lower than that of the two other groups pooled (Contrast II: $F_{1,16} = 7.13$, $p < 0.05$).

The choice correspondence increased over the trial blocks ($F_{5,80} = 18.07$, $p < 0.01$) to a similar degree in all three groups of rats (Trial blocks by Lesion interaction: $F_{10,80} = 1.67$, n.s.)

Choline acetyltransferase in the frontal cortex

Lesioning of the nbm affected the ChAT activity in the frontal cortex ($F_{2,36} = 46.6$, $p < 0.01$; see Fig. 5). There were no differences between the two control groups, i.e. the intact and the sham-lesioned rats (Contrast I: $F_{1,36} = 1.8$, n.s.). The nbm lesion reduced the ChAT activity in the frontal cortex by approximately 27%, when compared with that of the intact and the sham-lesioned groups (Contrast II: $F_{1,36} = 91.4$, $p < 0.01$).

Histological verification of the nbm-lesion

The size and location of the lesions were in good agreement with those seen in other experiments performed at our laboratory, in which the same lesion coordinates were used (see Chapter 3.2, Fig. 8, left panel for a schematic representation of the lesions, induced by 0.4 μ l ibotenic acid in the rats used in the present study to assess the effects of nbm lesions on ChAT activity).

Discussion

Bilateral lesioning of the nbm affected the performance of rats in a holeboard task. Both the WM and RM of the lesioned rats were impaired, compared with those of the intact control rats and the sham-lesioned rats. This finding supports the notion that the nbm has a role in both WM and RM (e.g. Murray & Fibiger, 1985; Wozniak et al., 1989; Givens & Olton, 1994). The results of the present study are strikingly similar to those found in an age-comparison study in which the spatial holeboard discrimination task was used (van der Staay, van Nies, and Raaijmakers, 1990). The differences between young and aged rats observed by van der Staay, van Nies, and Raaijmakers (1990) very closely parallel those between intact and sham-lesioned rats on the one hand, and nbm-lesioned rats on the other, found in the present study.

The lesion destroyed part of the nbm and resulted in a 27% decrease in ChAT activity compared with that of the intact and the sham-lesioned groups. This reduction in ChAT activity, assessed 7 weeks after lesioning, is within the range reported by others after ibotenic acid lesions of the nbm (e.g. 23-28%: Dokla & Thal, 1989; 31%: Robbins et al., 1989; 25-30%: Shaughnessy et al., 1994, 1996). In the sham-lesioned rats of the present study, the syringe had been lowered to DV 6.6, i.e. 1 mm above the lesion site (Wenk & Röskaeus, 1988; Wenk, Markowska & Olton, 1989), and then retracted in order to avoid mechanical damage to the target area. There was no difference between the intact and the sham-lesioned groups with respect to cortical ChAT activity. This finding is in agreement with results of other studies involving multiple controls, i.e. intact rats, and sham-lesioned rats with and without infusion of vehicle (e.g. Männistö et al., 1993; Grigoryan et al., 1994b; Waite & Thal, 1996). It is not clear whether the acute effects of lesioning of the nbm are stronger than those measured 7 weeks after operation. It is still a matter of debate whether cortical ChAT activity recovers over time, as

reported results are conflicting (Wenk & Olton, 1984; Thal, Dokla & Armstrong, 1988; Shaughnessy et al., 1996).

The WM performance of the nbm-lesioned group of rats differed from that of the other rats already at the start of the acquisition, whereas during formal training the three groups improved in parallel. This group difference may be because a WM procedure was applied during the habituation sessions: all holes were baited and revisits were not reinforced. Acquisition of the WM component may therefore have started during the habituation session, and the performance of the three groups may already have been different during this phase of the experiment (van der Staay, van Nies & Raaijmakers, 1990).

However, no differences were found between groups for the number of nose-pokes during adaptation sessions, a behavior that is considered as a measure for exploration (File & Fluck, 1994). The number of nose-pokes increased from the first to the second adaptation sessions in all groups, and no further change or a slight decrease was seen from the third to the fourth sessions. The increase from the first to the second sessions contrasts with findings reported by Voits and colleagues (1995), who observed a decrease in the number of nose-pokes on the second day of holeboard testing compared with the first day. They interpreted this reduction as habituation of nose-poke behavior. However, unlike the rats in the study by Voits et al. (1995), the rats in the present study were on a restricted feeding regimen, and food could be found in the holeboard apparatus. Motivational factors thus might have increased the number of nose-pokes in the second session. Extinction processes might have prevented a further increase in subsequent adaptation sessions, because once the pellets were eaten they were not replaced. Whatever processes might have modulated the changes in nose-poke behavior across adaptation sessions, they were not affected by the nbm lesions.

The nbm lesion-induced impairment in RM performance emerged as a function of formal training. The similar RM performance among the groups at the beginning of the formal training indicates that the animals visited the baited holes at chance level. During habituation no clues were provided as to which set of holes would be baited during formal training. Thus, whereas the acquisition of WM may already have started in the habituation sessions, the acquisition of RM did not.

Factors affecting WM and RM performance

Many factors may influence WM and RM performance. The speed of visiting the holes in the holeboard could interfere with choice accuracy and cannot be ruled out as a factor that may have contributed to differences between WM and RM performances. In all but the fourth trial block, the nbm-lesioned rats visited the holes much more slowly than the intact and the sham-lesioned rats, which never differed from one another. With longer inter-visit intervals, the list of hole visits within a trial must be retained in WM for longer. However, the duration of the inter-visit intervals declined across the first three trial blocks and reached a plateau in the fourth trial block, whereas the difference in RM performance between the groups continued to increase, and a stable difference in WM performance between the controls and the nbm-lesioned rats was found across the trial blocks. These three measures thus followed differently shaped curves across trial blocks.

The development of a response strategy for visiting the holes that contain food might facilitate performance in the spatial discrimination task (Hodges, 1996) because applying a strategy reduces the memory load, especially that of the WM. Oades and Isaacson (1978), for example, found that normal rats acquired their individual strategies to find food-containing holes. By contrast, hippocampus-lesioned rats (Oades & Isaacson, 1978; Oades, 1981a), and rats with lesions of the ventral tegmentum

(Oades, 1982) were impaired in the development of a search strategy and made more erroneous visits. Thus, intact and brain-lesioned rats might adopt different 'strategies' to solve spatial discrimination problems. For this reason we analyzed the similarity of the sequences of rewarded food-hole visits over subsequent trials as a potential source for the differences between the three groups.

A slight reduction in the variability of visiting the baited holes was found for all groups. However, the nbm-lesioned rats developed a less pronounced food search strategy than the other two groups did. Thus the food search pattern of the intact and the sham-lesioned rats could have contributed to their better memory performance. However, it cannot be excluded that the increase in 'choice correspondence' is not a cause but a consequence of the better performance of the intact and sham-lesioned rats. The differences between the three groups in choice correspondence, therefore, might be either a source or a consequence of the effects of the nbm lesion on the memory measures.

To summarize, the results of analyses of choice correspondence of reinforced visits and of inter-visit intervals do not support the notion that factors unrelated to 'cognitive processes' caused the differences in the WM and RM performance between the three treatment groups. Although we cannot completely rule out that other factors, such as the motivational state, could have contributed to the differences found between the intact and sham-lesioned rats on one hand, and the nbm-lesioned rats on the other, we conclude that bilateral lesioning of the nbm impairs spatial discrimination learning in the holeboard in rats.

3.2

Effects of bilateral ibotenic acid lesions of the nbm on the performance of rats in a seven-choice problem in a radial alley maze*

Abstract

We used a seven-choice task in an eight-arm radial alley maze, which has been found to be sensitive to age-associated impairments in learning and memory of rats, to study the influence of experimental lesions of the nucleus basalis magnocellularis (nbm) on spatial learning and memory in rats. The seven-choice task is a win-stay task and assesses spatial reference memory (RM). In the first experiment, bilateral lesioning of the nbm with ibotenic acid disrupted the acquisition of a seven-choice task in the radial alley maze. This finding was not replicated: in the third experiment bilateral lesions of the nbm at the same coordinates as in the first experiment had no effect on learning. A second set of lesion coordinates also had no effect.

In the second experiment, rats received nbm lesions after they had acquired the seven-choice task. The effects of the lesions on the retention, the acquisition of a new problem, and on re-acquisition of the originally acquired problem were tested. Under these conditions, the nbm lesions did not affect performance in the discrimination task.

Our results do not support the notion that cortical cholinergic activity originating in the nbm is critically involved in memory. However, it is possible that the lesion was too small to produce completed destruction of the nbm, or the task used was not sensitive enough to detect lesion-induced deficits, or a combination of both factors might have contributed to the inconclusive findings of our study.

Introduction

A pronounced degeneration of cholinergic pathways has been found in patients suffering from Alzheimer's disease (e.g. Coyle, Price & DeLong, 1983; Jacobs & Butcher, 1986; Davison, 1987; Sparks et al., 1992), and the resulting impairment of cholinergic neurotransmission is thought to be one of the major causes of age- and dementia-related cognitive impairments (cf. Hagan and Morris, 1988; Sahakian, 1988). Experimentally induced lesions of the nucleus basalis magnocellularis (nbm) and of the septo-hippocampal pathways have been used to study the role of central cholinergic neurotransmission in learning and memory processes in animals, as these lesions have been

* The results of the first experiment of this chapter have previously been reported as part of the publication: Raaijmakers, W.G.M., van der Staay, F.J., Drinkenburg, W.H.I.M. & Blokland, A. (1990). Age differences and effects of lesions in the nucleus basalis magnocellularis on a seven-choice task in a radial alley maze. In: van Bezooijen, C.F.A., Ravid, R. & Verhofstad, A.A.J. (Eds.), *From gene to man*. Rijswijk: Stichting Gerontologie en Geriatrie (ISBN 90-9003996-1), pp. 159-163.

suggested to mimic the mnemonic symptomatology seen in Alzheimer's patients (Kesner, Adelstein & Crutcher, 1987; Olton & Wenk, 1987).

We used a seven-choice task in an eight-arm radial alley maze, a task which is sensitive to age-associated impairments in learning and memory of rats (see also Chapter 3.3 and Appendix 6.2), to study the influence of lesions of the nbm on spatial learning and memory in rats. The seven-choice task is a win-stay task which measures spatial reference memory (RM), because the start arm and the correct goal arm, where the bait can be found, are the same across trials and sessions. Performance in this task is independent of locomotor ability, which makes the test especially suited to assess the performance of animals which, due to the experimental manipulations they underwent, might suffer from (subtle) motor impairments.

Three experiments were performed. In the first experiment, we assessed the effects of bilateral ibotenic acid-induced lesions of the nbm on the acquisition of a seven-choice spatial discrimination task. In the second experiment, rats acquired the seven-choice task before they received bilateral ibotenic acid lesions of the nbm. Then, we evaluated the effects of the lesion on retention, acquisition of a new task, and on re-acquisition of the originally acquired task. In the third experiment, we assessed the effects of two different lesion coordinates on the acquisition of the seven-choice task.

Experiment 1: effects of bilateral ibotenic acid lesions of the nbm on performance of a seven-choice spatial discrimination task in a radial alley maze

Material and Methods

Animals

Twenty male Wistar rats (Cpb:Wu) weighing approximately 280 grams were supplied by CPB, Zeist, the Netherlands. The rats were weight-matched and then semi-randomly assigned to an untreated ($n = 6$; mean body weight \pm SEM: 281.5 ± 4.4 grams), a sham-lesioned ($n = 6$; 279.3 ± 3.7 grams), or a nbm-lesioned ($n = 8$; 280.4 ± 3.1 grams) group. The experimental protocol is depicted in Table 1.

Table 1. Protocol of experiment 1, in which the effects of bilateral lesions of the nucleus basalis magnocellularis (nbm) on the acquisition of a seven-choice task in a radial alley maze were assessed. The week numbers (*: after arrival at our laboratory) and the treatments and testing procedures are listed.

Week*	Event
0	Arrival at our laboratory of 20 male Cpb:Wu rats Individual housing, reversed day/night cycle (lights on from 20:00 to 08:00)
2	Matching on body weight: assignment to control, sham-, or nbm-lesioned group Stereotaxic operations
3	Start of food deprivation and four adaptation sessions in the radial alley maze
4-6	Acquisition of seven-choice task in the radial alley maze
10	Removal of the brains, histological verification of lesion, biochemical assays

Apparatus

The eight-alley radial maze consisted of a central platform (diameter: 26 cm) from which 8 alleys radiated equidistantly (Fig. 1). The alleys were 46 cm long and 10 cm wide. The gray side walls were 38 cm high. All walls were made of gray polyvinyl chloride (PVC), except the end walls of the alleys, which were made of aluminum. A recess (5.0 cm * 4.0 cm * 4.7 cm) that contained a food tray was built about 2 cm above the floor into the end walls. The floor of the whole apparatus was made of Trespa®.

A cylindrical door (diameter: 49.5 cm, 16.6 cm above the floor when closed) that opened by moving down vertically until its upper edge reached the level of the floor of the apparatus allowed simultaneous access to the eight alleys. Hidden behind the closed door, about 5 cm above the floor of each alley, were black symbols on white screens (Munn, 1950) measuring 9.5 * 9.5 cm (Fig. 2). The screens could be illuminated, but the symbols were clearly visible whether or not the illumination behind the screens was switched on. When the door was open, the symbols provided distinct intra-maze cues in each alley.

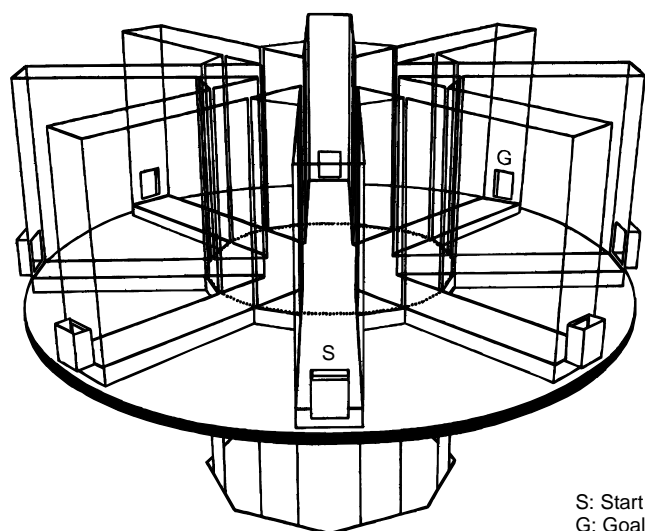


Figure 1. The eight-arm radial alley maze. The start alley and the goal alley are marked with 'S' and 'G', respectively. The dotted circle in the center of the maze delineates the position of the cylindrical door, which simultaneously gave access or blocked all arms, respectively, by moving down or up.

The experimenter controlled the opening and closing of the door. Visits to the alleys, operationalized as entering the alley by a rat's body length, and visits to the food cups were registered automatically by photosensitive cells. An Apple IIe microcomputer collected the data and controlled the duration of each trial. The radial alley maze was situated in a room that was illuminated by four red fluorescent strip lights and three 100-W bulbs, which were adjusted by a dimmer to provide illumination of about 50 lux at the floor of the apparatus.

The room had three doors, two one-way screens, and one window (screens and window covered with black curtains). Further, the room contained a sink, a table along one wall on which the computer and the interface were situated, and a holeboard apparatus. None of these potential extra-maze cues, however, were visible to the rat in the alley maze. The experimenter sat in a chair in front of the maze, and was not visible to the rat during testing.

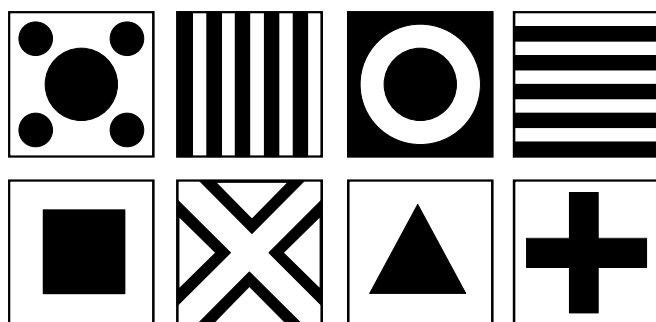


Figure 2. Symbols at the entrances of the eight alleys of the radial eight-arm alley maze. Hidden behind the closed door, about 5 cm above the floor of each alley, the symbols were depicted on white screens measuring 9.5*9.5 cm. When the door was open, the symbols provided distinct intra-maze cues for each of the eight alleys.

Surgical procedure

The animals received stereotaxically guided lesions in the nbm, for which a stereotaxic instrument (David Kopf) was used. The rats were anesthetized with a combination of Vetalar (i.m.: 50 mg/kg) and Rompun (s.c.: 2.25 mg/kg) (Guldin & Markowitsch, 1982). The bilateral nbm lesions were produced by ibotenic acid (4 µg in 0.4 µl phosphate buffer: pH 7.4) injected over 3.5 minutes at the coordinates: AP -0.5, L 2.3, and DV 7.6 (with respect to bregma and to the surface of the skull; Paxinos & Watson, 1986). The tip of the Hamilton syringe (28 gauge, needle point type 3) was left in place for 5 minutes and then the syringe was retracted. In the sham-lesioned rats, the syringe was lowered to DV 6.6 (1 mm above the lesion site; other coordinates as in nbm-lesioned group), but substances were not injected. The untreated rats were left undisturbed until behavioral testing began.

In the first 3 to 4 hours after surgery, the ibotenic-acid treated rats had seizures with profound arching of the back. We had observed in previous studies that during this period, the animals gnawed continuously, mostly their own forepaws (O'Connell, Earley & Leonard, 1994). To prevent this automutilation, we fitted the animals with a ruff which they wore for the first 8 hours after surgery (see Chapter 3.1, Fig. 1).

Behavioral testing

One week after surgery, all animals had regained or exceeded their pre-operation weights. The means (\pm SEM) were 312.4 grams (\pm 2.7) for the untreated rats, 291.2 grams (\pm 3.8) for the sham-lesioned rats, and 282.5 grams (\pm 3.9) for the nbm-lesioned rats. In the second week after surgery the body weights of all animals were gradually reduced to 85% of their free-feeding values. When the rats had reached their target weights, growth correction was applied, using the growth curves of undeprived male Wistar peers.

Adaptation sessions: the rats were familiarized with the radial alley maze on 4 consecutive days (10 min/day). These adaptation sessions started 24 hours after the rats had been put on the restricted feeding regimen. A rat was put in one alley (no. 1), then the circular door was opened and the animal could enter freely all parts of the apparatus. The food trays of each alley, except that of the start alley, were baited with one food pellet (45 mg, Campden Instruments). Additional pellets were scattered on the floor of the alleys and of the central platform. The start alley was distinct from the other seven alleys: at the entrance to the center of the apparatus the rats had to cross a low barrier (1.5 cm high).

Acquisition: when spatial discrimination testing in the seven-choice task began (1 week after the start of the adaptation sessions) all rats had reached their 85% target weight. During training, the rats were started from alley no. 1, and the alley 135° to the right of the start alley (no. 6; counted in clockwise

direction from alley no. 1) contained the food reward (four 45-mg food pellets). A trial was terminated as soon as the rat had found the food or when 10 minutes had elapsed, whichever event occurred first. Rats could enter and re-enter all alleys (including the start alley) freely during a trial. Thus, a correction procedure was applied. All rats were trained with massed trials (days 1 to 3: 2 trials/day; all successive days: 3 trials/day) until they had reached the criterion of seven faultless trials in a series of nine trials. The illumination of the cues in the alleys (Fig. 2) was switched off.

Dissection of brain samples

The rats were decapitated without anesthesia in the eighth week after surgery. The severed head was kept about 5 s in liquid nitrogen to cool the brain. The brain was then rapidly dissected at 4-10°C in an open refrigerator. The frontal cortex was dissected free. In the coronal plane the cortical sample was delimited with a calibrated plastic T-square (Rosenzweig, Bennett & Diamond, 1972; Raaijmakers, 1978), and the tissue under the area covered by the T-square was discarded (see Fig. 3). Rostrally, the frontal cortex sample was delimited by a horizontal knife-cut above the olfactory bulbi.

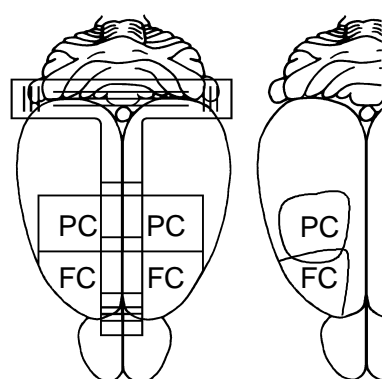


Figure 3. The calibrated plastic T-square (left panel) used to bilaterally dissect samples of the frontal cortex (FC; Chapters 3.1, 3.2, and 3.3) and parietal cortex (PC; Chapter 3.2, second and third experiment, and Chapter 3.3) of nucleus basalis magnocellularis (nbm)-lesioned rats. The approximate extensions and limits of these cortical areas according to Thompson (1978) are depicted in the right panel.

Choline acetyltransferase (ChAT) activity in the frontal cortex

ChAT activity in the frontal cortex sample was determined as described in Chapter 3.1.

Statistical analyses

Adaptation sessions: two measures of the adaptation sessions were analyzed statistically: number of alley visits and number of alleys visited.

- Number of alley visits is the total number of entries in the alleys of the maze during a 10-minute adaptation session. This measure comprises information about exploration and the speed of adaptation (changes in the number of alleys visited over adaptation sessions).
- Number of alleys visited is the number of alleys that were visited at least once during the adaptation sessions. The maximum score is 8. This measure indicates whether scanning and exploration of the apparatus are exhaustive.

Treatment effects on the adaptation sessions were analyzed by a one-factorial analysis of variance (ANOVA) in which the three lesion conditions (untreated, sham-lesioned, and nbm-lesioned) were considered as three levels of the factor Treatment. Changes in the course of the adaptation period

were assessed by a Session (sessions 1 to 4) by Treatment ANOVA with repeated measures on the first factor.

Planned comparisons contrasted:

- the untreated group with the sham-lesioned group (Contrast I); and
- the untreated and sham-lesioned groups pooled with the nbm-lesioned group (Contrast II).

Acquisition of the seven-choice task: two measures of formal training were analyzed statistically: trials to criterion and errors to criterion:

- Trials to criterion were the number of trials needed to reach the criterion of seven faultless trials in a series of nine trials. The criterion trials are included.
- Errors to criterion were the number of errors (visits and re-visits of the never baited alleys, including all visits to the start alley) made to reach criterion summed over all trials.

Four of the eight nbm-lesioned rats did not reach the criterion of seven faultless trials in a series of nine trials within 66 trials (censored data), when all rats of the untreated and sham-lesioned groups had already reached the criterion. Therefore, the treatment effects on the acquisition of the seven-choice task were analyzed non-parametrically, using Kruskal Wallis one-way analysis by ranks (Ferguson, 1971, pp. 331-333) in which the three lesion conditions (untreated, sham-lesioned, and nbm-lesioned) were considered as three levels of the factor Treatment. In addition, planned comparisons (multiple comparisons of mean ranks: Daniel, 1978) contrasted:

- the untreated group with the sham-lesioned group (Contrast I); and
- the untreated and sham-lesioned groups pooled with the nbm-lesioned group (Contrast II).

Results

Adaptation sessions

Number of alley visits (see Fig. 4, left panel): the lesions influenced the number of alley visits during the adaptation session. The nbm-lesioned rats entered the alleys more frequently than the rats of the other two treatment groups did during the adaptation sessions (General mean: $F_{2,17} = 5.0$; $p < 0.05$). There was no decrease or increase over adaptation sessions, as indicated by the repeated measures analysis (Session: $F_{3,51} = 1.8$, n.s.; Session by Treatment interaction: $F_{6,51} < 1.00$, n.s.).

Number of alleys visited: all groups of rats visited all alleys of the radial alley maze from the first adaptation session onward (results not shown).

Acquisition of the seven-choice task

Trials to criterion (see Fig. 4, center panel): the treatment affected the number of trials needed to reach criterion (Kruskal-Wallis: $X_2^2 = 12.48$, $p < 0.01$). Comparison of the performance of the untreated and sham-treated groups revealed that they needed a similar number of trials and errors to reach the criterion [Contrast I: critical delta (Δ_{crit}) = 6.69, observed delta (Δ_{obs}) = 2.58, n.s.]. The observed differences in mean ranks for trials to criterion of the pooled untreated and sham-lesioned groups versus the nbm-lesioned group (Contrast II: $\Delta_{crit} = 5.29$, $\Delta_{obs} = 9.26$, $p < 0.025$), on the other hand, exceeded the critical difference. Thus, the nbm lesion heavily impaired acquisition of the spatial seven-choice problem.

Errors to criterion (see Fig. 4, right panel): a similar picture was seen for the number of errors made until the criterion was reached. The treatment affected the errors to criterion (Kruskal-Wallis: $X_2^2 = 9.07$, $p < 0.01$). Comparison of the performance of the untreated and sham-treated groups revealed that they made a similar number errors before they reached criterion (Contrast I: $\Delta_{crit} = 6.69$, $\Delta_{obs} = 2.33$, n.s.). In contrast, the observed differences in mean ranks for errors to criterion between untreated and sham-lesioned pooled versus nbm-lesioned groups exceeded the critical differences (Contrast II: $\Delta_{crit} = 5.29$, $\Delta_{obs} = 7.92$, $p < 0.025$).

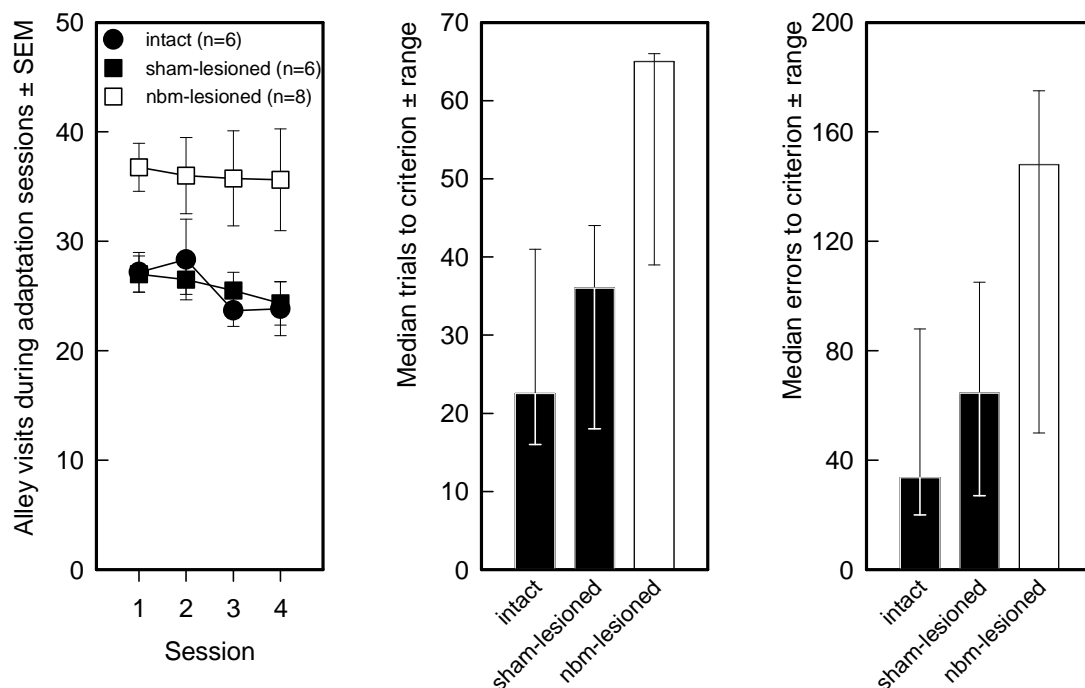


Figure 4. Mean number of alley visits \pm standard errors of the mean (SEM) during adaptation sessions 1 to 4 (left panel), and median and range of trials to criterion (center panel) and errors to criterion (right panel) of untreated, sham-lesioned, and nucleus basalis magnocellularis (nbm)-lesioned rats during acquisition of a seven-choice task in an eight-arm radial alley maze.

Choline acetyltransferase activity in the frontal cortex

The effects of the nbm lesion on ChAT activity in the frontal cortex are described in Chapter 3.1. The data of the present experiment were pooled with those of the holeboard experiment because the rats were approximately the same age, survival after lesioning was similar, and identical lesion coordinates had been used. In short, the nbm lesion reduced cortical ChAT activity by about 27% when compared with that of intact and sham-lesioned rats.

Experiment 2: effects of bilateral ibotenic acid lesions of the nbm on retention, acquisition of a new problem, and re-acquisition of the originally acquired problem in a seven-choice task

Material and Methods

Animals: twenty male Wistar rats (Cpb:Wu) weighing about 420 grams were supplied by CPB, Zeist, the Netherlands. The animals were housed in standard Makrolon cages and were habituated to a reversed day/night cycle (lights on from 20:00 to 08:00). The pre-operation weights were (mean \pm SEM): 449.7 (\pm 13.0) grams for the intact rats, 440.8 (\pm 15.1) grams for the sham-lesioned rats, and 449.5 (\pm 15.2) for the nbm-lesioned rats (all treatment groups: $n = 6$, due to the loss of two nbm-lesioned animals). There were no differences between the groups with respect to the pre-operation weights ($F_{2,15} = 0.16$, n.s.). The experimental protocol is summarized in Table 2.

Apparatus: the same apparatus was used as in experiment 1.

Table 2. Protocol of experiment 2, in which the effects of bilateral lesions of the nucleus basalis magnocellularis (nbm) on the performance of rats in a seven-choice task in a radial alley maze were assessed. The week numbers (*: after arrival at our laboratory) and the treatments and testing procedures are listed.

Week*	Event
0	Arrival at our laboratory of 20 male Cpb:Wu rats, weighing approximately 420 grams Individual housing Reversed day/night cycle (lights on from 20:00 to 08:00) Gradual reduction to 85% of the free-feeding body weight 10-minute adaptation sessions in the radial alley maze on four consecutive days
8	First acquisition of problem A (massed trials: 3 trials per day) to criterion; one additional session of 3 trials when the criterion was reached
11	Second acquisition of problem A (massed trials: 10 trials per day) to criterion Free access to food
15	Matching on trials to criterion problem A: assignment to control, sham-, or nbm-lesioned group Stereotaxic operations
18	First retention of problem A (massed trials: 10 trials per day)
19	Acquisition of problem B (massed trials: 10 trials per day)
19	Second retention of problem A (with massed trials in one session) Free access to food
20	Decapitation for biochemical assays

Surgical procedure: the animals received stereotaxically guided lesions in the nbm, as in the first experiment.

Behavioral testing

The body weights of all animals were gradually reduced to 85% of their free-feeding weights within about 1 week.

Adaptation sessions: the rats were familiarized with the radial alley maze on 4 consecutive days (10 min/day) as in the first experiment.

First and second acquisition of problem A: one week after the start of the adaptation sessions, all rats had reached their 85% target weight. The start position for problem A was alley no. 1; the goal alley (no. 6) with the food reward was 135° to the right of the start alley. The training procedure was as in the first experiment. In this phase of the experiment, the cues in the alleys were not illuminated.

All rats were trained with massed trials (3 trials/day) until they had reached a criterion of seven faultless trials in a series of nine trials (1st acquisition of problem A). Twenty-four hours after a rat had reached the criterion, it received three additional trials in one session. When all rats had reached the criterion and received the three extra trials, they were trained again to criterion (10 trials/day; 2nd acquisition of problem A). No rat needed more than two sessions to reach the criterion a second time. Between the first and second series to reach criterion the rats were kept on the food-restricted regimen. Then, the rats were returned to an ad-libitum feeding regimen for about 3 weeks. The animals were matched on trials to criterion (1st acquisition of problem A) and were semi-randomly assigned to one of three treatment conditions: untreated (n = 6), sham-lesioned (n = 6), and nbm-lesioned (n = 8).

After lesioning, the rats were allowed to recover from surgery for 2 weeks. By then, the animals had reached at least 95% of their pre-operation weights. One nbm-lesioned rat did not survive the anesthesia, and a second nbm-lesioned animal died during the recovery period. These losses reduced the number of nbm-lesioned animals to six. Taking into account the loss of these two nbm-lesioned rats, the pre-operation weights were (mean ± SEM): 449.7 (± 13.0) grams for the intact rats, 440.8 (± 15.1) grams for the sham-lesioned rats and 449.5 (±15.2) for the nbm-lesioned rats (all treatment groups: n = 6). There were no differences between the groups with respect to the pre-operation weights ($F_{2,15} < 1.0$, n.s.).

First retention of problem A: two weeks after lesioning, the weights of all animals were gradually reduced to 85% of their free-feeding weights within 1 week. Three weeks after the operation, retention of problem A was assessed with massed trials (10 trials/day) until the rats had reached the criterion of seven faultless trials in a series of nine trials. The illumination of the cues in the alleys was switched off.

Acquisition of problem B: in the fifth week after lesioning, the rats were trained to criterion on a new problem (problem B) with massed trials (10 trials/day). Now, rats started from alley no. 7 and food could be found in alley no. 2, 135° to the left of the start alley. This time, the cues in the alleys were illuminated.

Second retention of problem A: after completion of training on problem B, all rats were again trained on problem A to criterion within one single session. The cues in the alleys were not illuminated, as had been the case during the first acquisition of problem A.

Dissection of brain samples: the procedure followed was as in the first experiment. However, two cortical samples, the frontal and the parietal cortices were dissected free. In the coronal plane, the cortical samples were delimited using a calibrated plastic T-square (Rosenzweig, Bennett & Diamond,

1972; see Fig. 3), with the tissue under the areas covered by the T-square being discarded. Rostrally, the frontal cortex sample was delimited by a horizontal knife-cut above the olfactory bulbi.

Choline acetyltransferase (ChAT) activity in the frontal and parietal cortex: ChAT activity in the frontal and parietal cortices was determined and analyzed statistically as described in Chapter 3.1.

Statistical analyses

Adaptation sessions: the number of alley visits and number of alleys visited were analyzed as in experiment 1.

First and second acquisition of problem A, first retention of problem A, acquisition of problem B, and second retention of problem A: treatment effects on the two measures, trials and errors to criterion (see experiment 1 for details), were analyzed by a one-factorial (Treatment) ANOVA for each of the five phases of the experiment. The three lesion conditions (untreated, sham-lesioned, and nbm-lesioned) were considered levels of the factor Treatment. The analyses were supplemented with planned comparisons.

Results

Adaptation sessions

Number of alley visits (see Fig. 5, left panel): there were no differences between the treatment groups during the adaptation sessions, except in the first session ($F_{2,15} = 3.85$, $p < 0.05$). Planned comparisons revealed that the rats in the untreated (intact) group made more alley visits than the rats in the sham-lesioned condition (Contrast I: $F_{1,15} = 7.22$, $p < 0.05$). An additional comparison contrasting the alley visits of the sham-lesioned rats with those of the nbm lesion-lesioned rats revealed that these two groups of animals did not differ ($F_{1,15} < 1.00$, n.s.).

The repeated measures analysis revealed that treatment groups did not differ for the number of alley visits averaged over all adaptation sessions (General mean: $F_{2,15} = 0.92$, n.s.). The number of alley visits changed over sessions ($F_{3,45} = 4.75$, $p < 0.01$), but this change was similar for the three groups (Session by Treatment interaction: $F_{6,45} = 1.32$, n.s.).

Number of alleys visited: the rats visited all alleys of the radial alley maze from the second adaptation session onward (results not shown).

Acquisition and retention sessions

First and second acquisition of problem A: the results are summarized in Fig. 5, center and right panel. There were no differences between the treatment groups for the number of trials and errors to reach the criterion of seven faultless trials in a series of nine trials twice before lesioning (all $F_{2,15} < 1.0$, n.s.). These results confirm that matching on trials to criterion was successful, despite the fact that two nbm-lesioned rats died.

First retention of problem A: the nbm lesion had no effect on the number of trials ($F_{2,15} = 1.12$, n.s.) and errors ($F_{2,15} = 1.29$, n.s.) to reach the criterion. There was, however, a strong increase in the variance in the nbm-lesioned group. Therefore, the data were re-analyzed using the non-parametric Kruskal-Wallis one-way analysis of variance by ranks (Ferguson, 1971, pp. 331-333). The non-parametric analyses corroborated the results of the ANOVAs (trials to criterion: $H_2 = 1.27$, n.s.; errors to criterion: $H_2 = 0.32$, n.s.).

Acquisition of problem B: again, nbm lesions had no effect on the number of trials ($F_{2,15} = 2.52$, n.s.) and errors ($F_{2,15} = 3.55$, $0.10 > p > 0.05$) to criterion. The F-ratios for treatment effects on errors to criterion and the first planned comparison (Contrast I: intact versus sham-lesioned) for trials ($F_{1,15} = 3.87$, $0.10 > p > 0.05$) and errors ($F_{1,15} = 4.39$, $0.10 > p > 0.05$) to criterion had associated probabilities close to 0.05. We used the planned comparisons I and II to detect specifically the effects of nbm lesions. These comparisons were, however, less suited to detect general effects of the operations. Therefore, we supplemented the analyses with two additional comparisons, which contrasted:

- trials and errors of sham-lesioned rats with that of those of nbm-lesioned rats (Contrast III); and
- trials and errors of intact animals with those of sham-operated and nbm-lesioned rats pooled (Contrast IV).

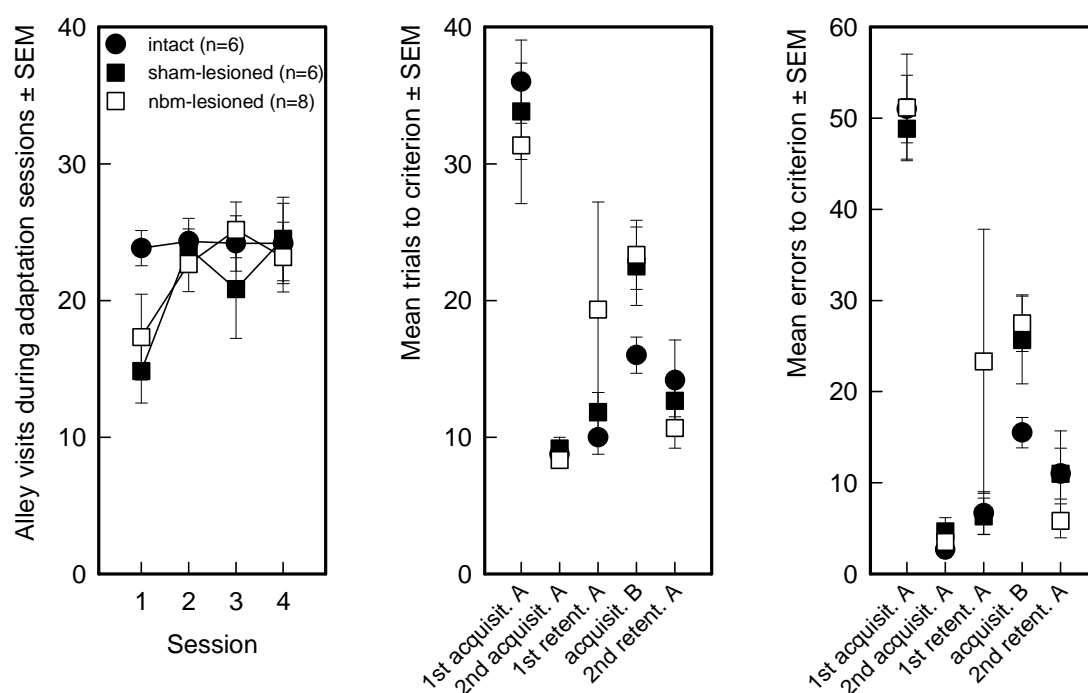


Figure 5. Number of alley visits during adaptation sessions 1 to 4 (left panel), trials to criterion (center panel) and errors to criterion (right panel) of untreated, sham-lesioned, and nucleus basalis magnocellularis (nbm)-lesioned rats during the first and second acquisition of problem A, first retention of problem A, acquisition of problem B, and second retention of problem A. The means and standard errors of the means (SEM) are shown.

These comparisons revealed that trials and errors to criterion were similar for the sham-operated and the nbm-lesioned rats (Contrast III: $F_{1,15} < 1.00$, n.s., for trials to criterion; $F_{1,15} < 1.00$, n.s., for errors to criterion). The two operated groups pooled needed more trials to reach the criterion and made more errors than the intact animals (Contrast IV: $F_{1,15} = 5.03$, $p < 0.05$, for trials to criterion; $F_{1,15} = 6.95$, $p < 0.05$, for errors to criterion). Thus, the stereotaxic operation *per se* might have impaired acquisition of a new problem.

Second retention (re-acquisition) of problem A: neither trials nor errors to criterion were affected by the nbm lesions.

ChAT activity in the frontal and parietal cortices: lesioning of the nbm affected the ChAT activity in the frontal cortex ($F_{2,15} = 24.01$, $p < 0.01$), and parietal cortex ($F_{2,15} = 9.28$, $p < 0.01$; see Fig. 6). There were no differences between the two control groups, i.e. the intact and the sham-lesioned rats (Contrast I for both cortex samples: $F_{1,15} < 1.00$, n.s.). The nbm lesion reduced ChAT activity in the frontal cortex by approximately 32.8%, in the *parietal* cortex by 22.9%, when compared with those of the intact and the sham-lesioned groups pooled (Contrast II for *frontal* cortex: $F_{1,15} = 48.00$, $p < 0.01$; Contrast II for *parietal* cortex: $F_{1,15} = 18.20$, $p < 0.01$).

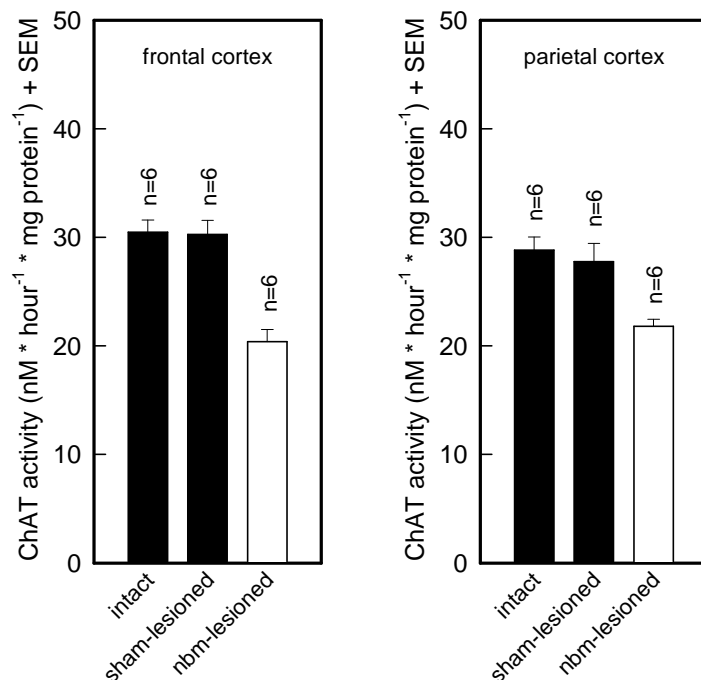


Figure 6. Effects of lesioning of the nucleus basalis magnocellularis (nbm) in adult Wistar rats. The mean ChAT activity in the frontal cortex (left panel) and in the parietal cortex (right panel), calculated as the nanomoles acetylcholine formed per milligram protein per hour, and the standard errors of the means (SEM) are shown for intact, sham-lesioned, and nbm-lesioned rats. The group sizes are depicted in the figure.

Experiment 3: bilateral ibotenic acid lesions of the nbm: effects of different lesion coordinates

Material and Methods

Animals: twenty male Wistar rats (Bor:WISW(SPF Cpb) weighing approximately 425.5 ± 4.6 grams (mean \pm SEM) were supplied by Winkelmann (Borchen, Federal Republic of Germany). The rats were weight-matched and then semi-randomly assigned to a sham-lesioned ($n = 8$) or a nbm-lesioned ($n = 12$) group. Within these groups, half of the animals were assigned to one of two lesion coordinate conditions.

Apparatus: the same eight-arm radial alley maze was used as in experiments 1 and 2.

Surgical procedure: the animals received stereotactically guided lesions in the nbm, using the stereotaxic instrument developed by Lohman and Peters (1976). The rats were anesthetized with a combination of Vetalar (i.m.: 50 mg/kg) and Rompun (s.c.: 2.25 mg/kg) (Guldin & Markowitsch, 1982). Two sets of coordinates were used to lesion the nbm. Half of the sham-lesioned animals (sham lesion A: $n = 4$) and half of the nbm-lesioned animals (nbm lesion A: $n = 6$) received injections of the toxin or

vehicle at the coordinates (AP -0.5, L 2.3, DV 7.6) with respect to bregma (Paxinos & Watson, 1986). The ventral coordinates were determined with respect to the surface of the skull. For the other sham-lesioned (sham lesion B, n = 4) and nbm-lesioned animals (nbm lesion B, n = 6) the coordinates were AP -0.8, L 2.7, DV 8.0.

Ibotenic acid (4 µg in 0.4 µl phosphate buffer: pH 7.4) or phosphate buffer alone (0.4 µl) was slowly injected over 4 minutes. The tip of the Hamilton syringe was kept in place for 1 minute and then the syringe was retracted.

Behavioral testing

Adaptation sessions: in the third week after surgery, the weights of all animals were gradually reduced to 85% of their free-feeding values. The rats were familiarized with the radial alley maze on 4 consecutive days as in experiments 1 and 2.

Acquisition: when spatial discrimination in the seven-choice task began (1 week after the start of the adaptation sessions), all rats had reached their 85% target weight. Testing was similar to the procedure described in experiment 1. All rats were trained with massed trials (day 1: 2 trials; days 2 to 5: 4 trials/day; days 6 and 7: 6 trials/day; all successive days: 8 trials/day) until they had reached a criterion of seven faultless trials in a series of nine trials.

Choline acetyltransferase (ChAT) activity: ChAT activity was determined as in the previous experiment.

Dissection of brain samples: the frontal and the parietal cortices were dissected as described previously (exp. 2). Then the remaining neocortex and corpus callosum were removed, and the hippocampus was dissected free. The dorsal part was separated *in situ* from the rest of the hippocampus by a knife-cut perpendicular to the ventro-dorsal extension of this structure, starting at the junction between the inferior and superior colliculi. Adhering white matter was removed from all samples.

Statistical analyses

Adaptation sessions: the number of alley visits and the number of alleys visited during the four adaptation sessions were analyzed by a Lesion (pooled sham-operated, vs. nbm-lesioned using coordinates A, vs. nbm-lesioned using coordinates B) by Sessions (adaptations sessions 1 to 4) ANOVA, with repeated measures on the last factor.

Acquisition: the same two measures as in the previous experiments were analyzed: trials and errors to criterion. The effects of nbm lesions on the two measures were analyzed by an ANOVA with the factor Lesion.

Cortical and hippocampal ChAT activity: an analysis of variance with the factor Lesion on the ChAT activity of the frontal and parietal cortices and of the dorsal hippocampus was performed [SAS general linear model (GLM) procedure for unequal cell sizes, Freund & Littell, 1985].

These ANOVAs were supplemented with three planned comparisons, which contrasted:

- the pooled sham-lesioned groups with the group of rats receiving nbm lesions at the first set of coordinates (Contrast I);
- the sham-lesioned groups with the group of rats receiving nbm lesions at the second set of coordinates (Contrast II);

- the nbm-lesioned group with lesions at the first set of coordinates (nbm lesion A) with the nbm-lesioned group of rats receiving nbm lesions at the second set of coordinates (nbm lesion B) (Contrast III).

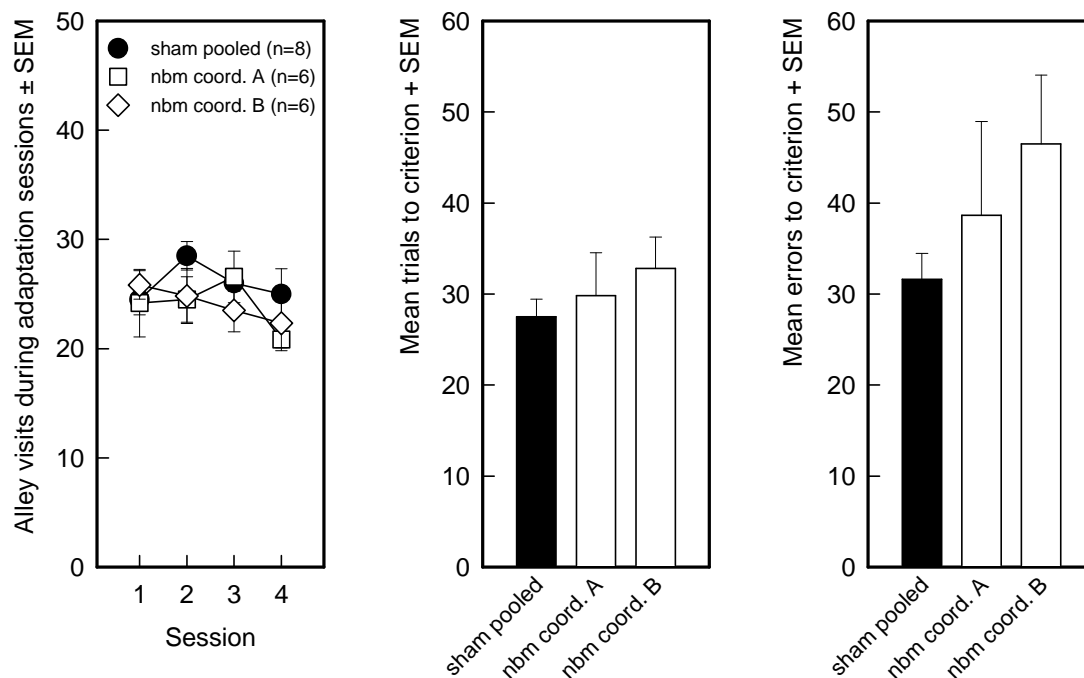


Figure 7. Number of alley visits during adaptation sessions 1 to 4 (left panel), trials to criterion (center panel) and errors to criterion (right panel) of sham-lesioned and nucleus basalis magnocellularis (nbm)-lesioned rats. Two different sets of coordinates (A, B) were used to induce the lesions. The means and standard errors of the means (SEM) are shown.

Results

Body weights (data not shown)

The rats in the different groups weighed the same before surgery ($F_{2,17} < 1.00$, n.s.), and 2 weeks after surgery, the rats had regained and exceeded their pre-operation weight. The nbm lesion did not affect body weight (Lesion: $F_{1,17} = 2.13$, n.s.).

Behavioral testing

Adaptation sessions

Number of alley visits (see Fig. 7, left panel): lesioning of the nbm did not affect the number of alley visits during the adaptation session, averaged over the four sessions (General mean: $F_{2,17} < 1.00$, n.s.). The number of alley visits changed slightly across sessions ($F_{3,51} = 3.80$, $p < 0.05$), but similarly for the three groups (Lesion by Sessions interaction: $F_{6,51} = 1.75$, n.s.)

Number of alleys visited: the rats visited all alleys of the radial alley maze from the first adaptation session on (results not shown).

Acquisition of the seven-choice task

Lesioning of the nbm did not affect the number of trials ($F_{2,17} = 1.19$, n.s.; Fig. 7, center panel) and errors to criterion ($F_{2,17} < 1.00$, n.s.; Fig. 7, right panel) differently.

ChAT activity in the dorsal hippocampus, the frontal and parietal cortices

The effects of the lesions on the ChAT activity of the frontal and parietal cortices and of the dorsal hippocampus are summarized in Fig. 8.

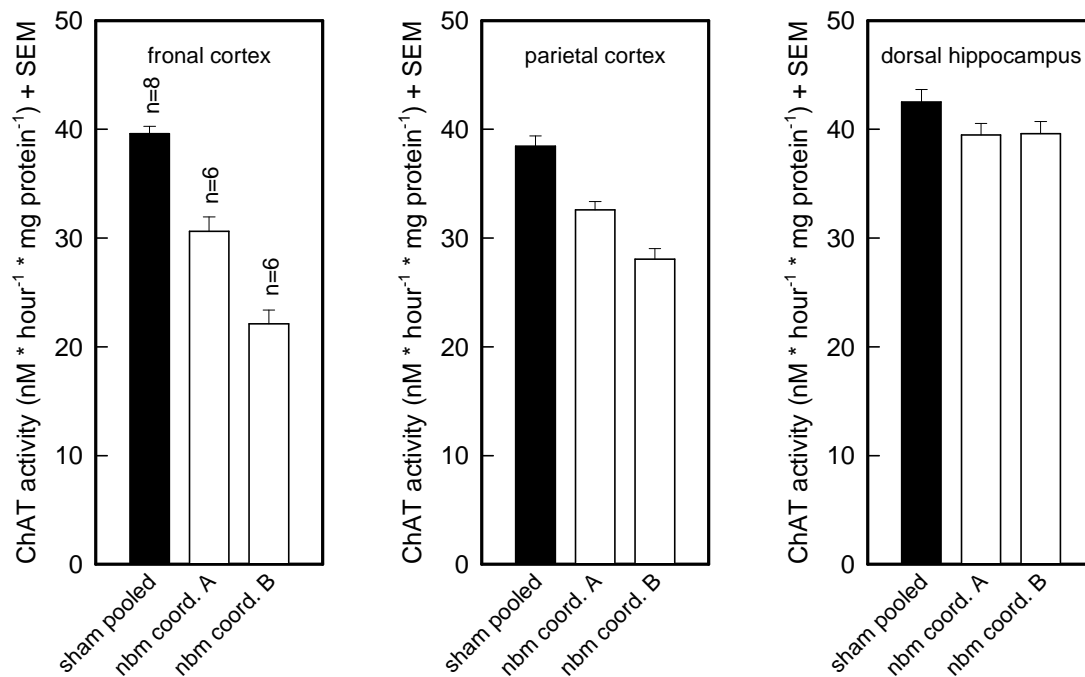


Figure 8. ChAT activity in the frontal cortex (left panel), the parietal cortex (center panel) and the dorsal hippocampus (right panel) of the pooled sham-lesioned and the nucleus basalis magnocellularis (nbm)-lesioned rats. Two different sets of coordinates (A, B) were used to induce the nbm lesions. The number of animals per group is depicted in the left panel. The means and standard errors of the means (SEM) are shown.

Frontal cortex: although both nbm lesions reduced the ChAT activity in the frontal cortex ($F_{2,17} = 71.00$, $p < 0.01$, and planned comparisons I and II; Contrast I: $F_{1,17} = 37.04$, $p < 0.01$; Contrast II: $F_{1,17} = 140.77$, $p < 0.01$), the nbm lesion B was more effective than the nbm lesion A in decreasing ChAT activity (Contrast III; $F_{1,17} = 29.21$, $p < 0.01$). Compared with the pooled sham-lesioned controls, the rats with the nbm lesion A had a 22.6% reduction in ChAT activity 6 weeks after surgery, and rats with the nbm lesion B a 44.2% reduction.

Parietal cortex: the nbm lesions reduced the ChAT activity in the parietal cortex ($F_{2,17} = 33.91$, $p < 0.01$, and planned comparisons I and II; Contrast I: $F_{1,17} = 21.21$, $p < 0.01$; Contrast II: $F_{1,17} = 66.29$, $p < 0.01$) by 21.2%, compared with that of the pooled sham-lesioned rats. Animals with the nbm lesion A had a 15.3% reduction of ChAT activity compared with a 27.1% reduction in rats with nbm lesion B (Contrast III: $F_{1,17} = 10.95$, $p < 0.01$).

Dorsal hippocampus: the nbm lesions did not affect the ChAT activity in the hippocampus (Lesion: $F_{2,17} = 2.45$, n.s.).

Histological verification of the lesions

The location of the lesions in the nbm was verified histologically (Fig. 9). The schematic representation is based on the brains of 15 rats which received bilateral ibotenic acid injections at the coordinates AP -0.5 (with respect to bregma), L 2.3 (with respect to the midline), and DV 7.6 (with respect to the skull), and on the brains of 6 rats which received ibotenic acid injections at the coordinates AP -0.8, L 2.6, and DV 8.0. As already noted by Wenk, Cribbs, and McCall (1984), the contours of the lesions appeared to follow natural hydrophobic myelinated borders. They suggest that these structures in fact might guide the distribution of ibotenic acid. In sham-lesioned rats, no damage was found.

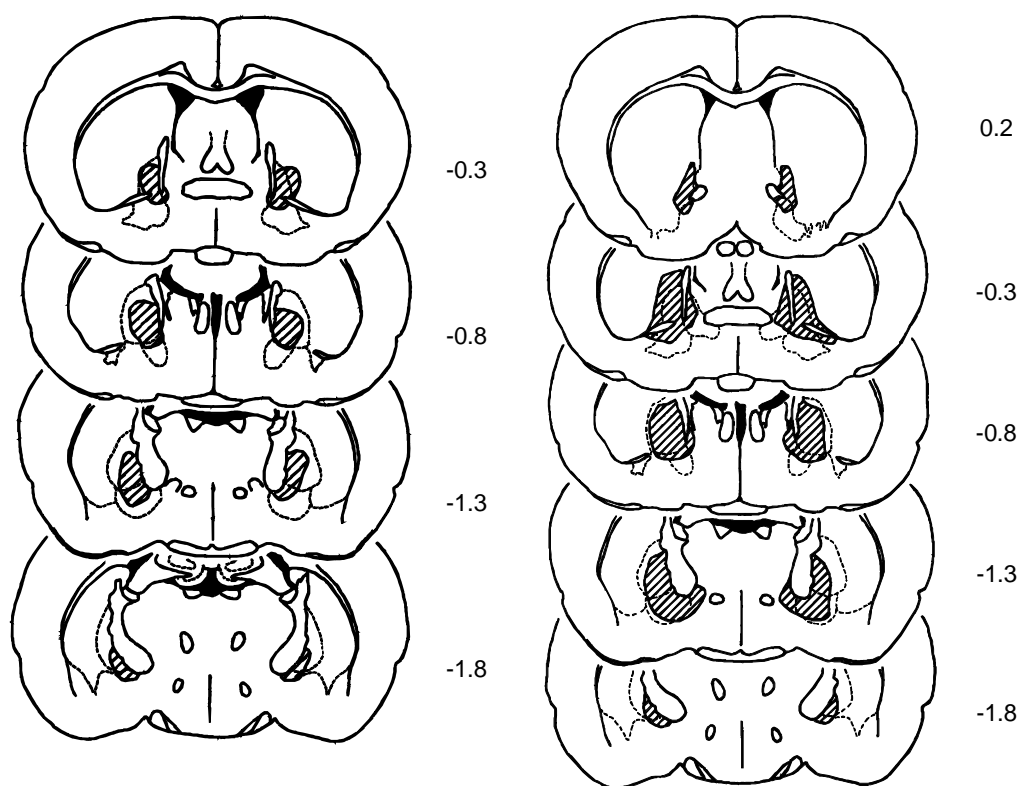


Figure 9. Schematic representation of the size and position of nucleus basalis magnocellularis lesions in cresyl-violet stained slices. The area of cell loss is indicated by shading. The left series of slices represents the composites of the brains of 15 rats which received bilateral injections of 0.4 μ l ibotenic acid at the lesion coordinates AP -0.5, L 2.3, DV 7.6. The series depicted on the right is based on the slices from 6 rats which received bilateral injections of 0.4 μ l ibotenic acid at the lesion coordinates AP -0.8, L 2.7, and DV 8.0. The AP-coordinates are with respect to bregma (Paxinos & Watson, 1986). The dorsoventral coordinates are determined with respect to the surface of the skull.

Discussion

In the first experiment, bilateral lesioning of the nbm disrupted the acquisition of a seven-choice task in the radial alley maze. This finding was not replicated in the third experiment: lesioning of the nbm at the

same set of coordinates as in the first experiment had no effect on learning. A second set of lesion coordinates also had no effects.

In the second experiment, rats received nbm lesions after they had acquired the seven-choice task. Then, the effects of the lesions on the retention, the acquisition of a new problem, and on the re-acquisition of the originally acquired problem were tested. Under these conditions, the nbm lesions did not affect rat's performance in the discrimination task.

Effects of nbm lesions on spatial discrimination performance

The seven-choice task, similar to the stem-discrimination component of the T-maze task of Wenk, Markowska, and Olton (1989) and the win-stay task in the T-maze of Liljequist et al. (1997), is considered to assess spatial RM. Both the T-maze and the seven-choice task consist of a start alley, at the end of which choice alternatives are provided. However, the seven-choice task is probably more complex than T-maze tasks, because it provides seven alternative choices, only one of which is correct.

Liljequist and colleagues (1997) found that ibotenic acid-induced nbm lesions did not affect performance in the T-maze in which choosing the left arm (win-stay) was always reinforced with food. The injection coordinates used by Liljequist and co-workers (1997) were similar to those used in our study. The injection volume, however, was 1 μ l per side, compared with 0.4 μ l in our experiments.

In a T-maze alternation task, which consists of two components, a stem discrimination and an arm discrimination, Wenk, Markowska, and Olton (1989) compared the effects of bilateral nbm lesions induced with quisqualic or ibotenic acid. Stem discrimination might be considered as an index of spatial RM, whereas arm discrimination might provide a measure of spatial working memory (WM; Barnes et al., 1990). The lesion coordinates were similar to those used in our study. However, injections were made at two sites, with an injection volume of 0.5 μ l per site. The ibotenic acid lesion induced a transient deficit in stem discrimination (RM), whereas quisqualic acid did not affect this component of the task. Ibotenic acid lesions of the nbm induced a permanent deficit in arm discrimination (WM), whereas the quisqualic acid-induced lesions affected arm discrimination only transiently.

The performance of rats with combined ibotenic acid-induced lesions of the medial septal area (msa) and the nbm was found to be impaired in a serial reversal of a two-choice spatial discrimination task in a T-maze (Peternel et al., 1988) similar to that used by Wenk, Markowska, and Olton (1989). In this task, the rats were trained to a criterion of 14 correct choices in a series of 15 trials to visit one arm of the maze in order to obtain a food reward. Then, the other arm was the correct choice until the rat had again reached the criterion. Multiple reversals were given. The nbm-lesioned rats reached criterion in all reversals, but they always needed more trials than the sham-lesioned rats. Contrary to the findings by Wenk and co-workers (1989), Peternel and colleagues (1988) found no differences between the groups on stem discrimination.

Connor and colleagues (1993) found that bilateral injections of ibotenic acid into the nbm (two injections per side) impaired non-matching to sample in a T-maze, a WM task. In this task, one arm of the maze was blocked during a forced run, followed by a free run in which both arms were open. The arm opposite to the arm of the forced run was baited with a food reward. The nbm-lesioned rats needed more trials to reach criterion than the sham-lesioned controls.

Waite and Thal (1996) either produced combined excitotoxic lesions (ibotenic or quisqualic acid, or AMPA) of the nbm and msa, or destroyed the cholinergic cells in hippocampus and cortex, the main projection fields of the msa and the nbm, respectively, by intra cerebroventricular infusion of the

immunotoxin 192 IgG-saporin. The bilateral excitotoxic lesions of the nbm were produced by a series of four injections per side. Four additional injections per side were aimed at the msa and the diagonal band. Under these conditions, all lesions produced deficits in the acquisition of a Morris water escape task. The ChAT activity in the frontal and parietal cortices and the hippocampus after the ibotenic acid lesions was decreased by 62%, 79%, and 56%, respectively.

Wirsching et al. (1989) trained rats until they had reached a stable performance in a radial arm maze task, which allows the assessment of WM and RM simultaneously. Then the nbm was lesioned unilaterally with 1 µl of quinolinic acid (120 nmol). The injection coordinates were similar to those we used. Both, the WM and the RM were permanently disrupted by the lesion.

Kinoshita et al. (1992) observed an impaired acquisition of the Morris water escape response by rats with bilateral ibotenic acid lesions (0.5 µl ibotenic acid was injected on each side). This task measures spatial RM.

From the above, it can be seen that heterogeneous results have been reported. Neither the task demands nor the lesions can directly be compared between studies. With respect to the lesions, a multitude of coordinates, neurotoxins and immunotoxins have been used. Ibotenic acid lesions were found to impair spatial RM, measured in the T-maze and in the Morris maze. However, the deficits occurred only after injection of 1 µl, after multiple injections of the neurotoxic compound, or when the nbm lesions were combined with lesioning of additional forebrain areas. In addition, the negative results of the study by Liljequist and colleagues (1997) with the simple win-stay task in the T-maze point to the possibility that the task must exceed a certain degree of complexity to become sensitive to the cognition-disrupting effects of nbm lesions. In this respect, the seven-choice task appears to be of 'borderline sensitivity', i.e. its sensitivity seems to be insufficient to detect nbm-lesion induced deficits reliably. Olton and Schlosberg (1978), in their comparison of behavior in win-stay and win-shift tasks using the elevated eight-arm radial maze, concluded that rats are disposed to follow a win-shift strategy and that they show severely retarded learning if a win-stay strategy is required to solve the task.

Influences of the lesions site and size

The lesions in the nbm were quite small: we injected 0.4 µl ibotenic acid and probably destroyed only a part of the cells in the nbm. However, Wenk, Cribbs, and McCall (1984) found that lesions induced by a smaller injection volume (0.6 µl) destroyed more cells in the target area than a larger volume (1.0 µl) did, even though equimolar amounts of the toxin were infused. In most studies on the behavioral consequences of nbm lesions, the volume of neurotoxin, such as ibotenic, quisqualic, or quinolinic acid, injected was larger than the volume we injected. The most frequently used volume to induce nbm lesions is 0.5 µl (e.g. Stone et al., 1989; Shaughnessy et al., 1994), but larger volumes have also been injected (e.g. 1 µl: Zawia, Arendash & Wecker, 1992; Liljequist et al., 1997; 1.5 µl: Ohara et al., 1997). In many studies, multiple injections of neurotoxins were given to destroy the nbm (e.g. two injections: Zawia, Arendash & Wecker, 1992; Connor et al., 1993; three injections: Steckler et al., 1993), or to destroy both the nbm and the msa with multiple injections (e.g. Grigoryan et al., 1994a; Hodges et al., 1995; Robinson et al., 1996) in an attempt to induce maximum damage to the cholinergic forebrain system. With larger injection volumes and multiple injections, the probability of lesion-induced behavioral deficits seems to increase.

The size and placement of the nbm lesions we induced might have induced 'threshold' damage, which sometimes does, and sometimes does not, lead to behavioral impairments. However, the lesion spared adjacent areas such as the diagonal band of Broca (dbB) and the msa. These two cell groups

provide cholinergic projections to the hippocampus, a structure that is critically involved in (spatial) learning and memory (Jarrard, 1993, 1995; Bunsey & Eichenbaum, 1996). The dbB might even account for 60% of the hippocampal cholinergic input (Wenk, Cribbs & McCall, 1984). The absence of effects of the nbm lesion on ChAT activity in the hippocampus, which was confirmed for both sets of our injection coordinates, supports the notion that neither the dbB nor the msa were damaged. The lack of remote damage after ibotenic acid-induced lesions, as compared with lesions induced by kainic acid, has already been described by Schwarcz and colleagues (1979) and by Guldin and Markowitsch (1981, 1982).

Across the three experiments, the lesions induced a 22% to 44% reduction in ChAT activity in the frontal cortex. In the parietal cortex, the reduction ranged from 15% to 27%, when compared with the respective controls, i.e. the groups which were not lesioned or which had received a sham lesion. These decreases in cortical ChAT activity are well within the range reported by others (e.g. 23-28%: Dokla & Thal, 1989; 31%: Robbins et al., 1989; 25-30%: Shaughnessy et al., 1994; Shaughnessy et al., 1996; 26%: Liljequist et al., 1997). Experiment 3 showed that lesioning of the nbm at the coordinates B (AP -0.8, L 2.7, and DV 8.0) was more effective in reducing cortical ChAT activity than lesions induced at coordinates A (AP -0.5, L 2.3, DV 7.6). This difference in effectiveness to reduce cortical ChAT, however, was not mirrored by a differential effect on the behavioral measures.

A factor which might have affected the decrease in ChAT activity is the survival time after lesions. It is not clear whether ChAT activity in the cortex recovers after lesioning of the nbm. Wenk and Olton (1984), for example, reported complete recovery of ChAT activity after unilateral ibotenic acid lesion of the nbm within 3 months. A similar recovery was seen in a study by Robinson and colleagues (1996): 18 weeks after the lesion, cortical ChAT activity was similar in sham-lesioned and ibotenic acid-lesioned rats. By contrast, no recovery of cortical ChAT activity up to 3 months after bilateral nbm lesions was seen by Thal, Dokla, and Armstrong (1988). Reviewing the results on the decreases in ChAT activity in relation to the time elapsed after the lesion, Dekker, Connor, and Thal (1991) concluded that most studies did not find evidence for recovery.

A problem inherent to lesion studies is the choice of optimal lesion coordinates to induce cell loss in the target area, while sparing adjacent structures which might induce behavioral deficits by themselves. Wenk, Cribbs, and McCall (1984) identified the optimal coordinates for nbm lesions using ibotenic acid. The lesion coordinates we used are within their range of coordinates found to induce maximal reduction of ChAT activity in the frontal neocortex.

Small lesions in this respect might be more selective than large ones. However, the magnitude of the damage might be too small to induce behavioral effects. In contrast, bigger lesions, or series of lesions to destroy the entire nbm, might damage too much of the surrounding structures, resulting in nonspecific behavioral deficits, or behavioral impairments specific for the adjacent structure. In this case, the specific effects of lesioning the nbm cannot be distinguished from those caused by damage to surrounding structures.

Recently, the validity of these experimentally induced deficits in learning and memory as an animal model for Alzheimer's disease or of specific symptoms of this disease has been questioned (Fibiger, 1991; Dunnett, Everitt & Robbins, 1991). However, notwithstanding the problems associated with the nbm lesion model and the neurotoxins used to induce it, this model is still frequently used to test, for example, the effects of putative cognition enhancers (e.g. Riekkinen, Riekkinen & Sirviö, 1992; Männistö et al., 1993; Itoh et al., 1997; Liljequist et al., 1997) and neuroprotective compounds (e.g. Männistö, et al, 1993; O'Connell, Earley & Leonard, 1994).

Our results do not support the notion that the cortical cholinergic activity originating in the nbm is critically involved in memory. Two main reasons may account for this result. First, the lesion might have been too small, and thus the damage induced incomplete. Second, the task used might not have been sensitive enough to detect lesion-induced deficits; however, a combination of both factors might also have contributed to the inconclusive findings of the present study. The lesion-induced reduction in cortical ChAT activity that we measured was well within the range that has been reported to cause impairments in cognitive performance.

3.3

Behavioral effects of bilateral lesions of the nbm, induced by ibotenic acid, in young adult and old Wistar rats

Abstract

Lesioning of the nucleus basalis magnocellularis (nbm) in rodents, the analogue of the nucleus basalis of Meynert in humans, has been suggested as an animal model for Alzheimer's dementia. This nucleus, which provides the major cholinergic input to the neocortex, undergoes massive cell loss in patients suffering from Alzheimer's disease. It is conceivable that lesioning of the nbm in aged rodents provides a model of Alzheimer's disease that shares more aspects of the Alzheimer symptomatology than lesioning of the nbm of young rodents would do. In order to test this hypothesis and to assess the effects of aging, of ibotenic acid-induced lesions of the nbm, and of the interaction between age and lesion, we tested young and aged Wistar rats in a battery of behavioral tests. The battery consisted of a seven-choice task in an eight-arm radial alley maze and a series of sensorimotor tests. We found clear age-associated impairments in sensorimotor tests and in the acquisition of the seven-choice task. Lesioning of the nbm did not affect the performance of the rats in the sensorimotor tasks used. The nbm lesion had a transient effect on the acquisition of the seven-choice task. The nbm-lesioned rats made more errors before they reached criterion, but all rats eventually acquired this discrimination task. The effects of the lesion were similar in both age groups. We could not confirm our hypothesis that aged, nbm-lesioned rats would provide a better model of Alzheimer's dementia than young nbm-lesioned rats would.

Introduction

Lesioning of the nucleus basalis magnocellularis (nbm) in rodents has been suggested as an animal model for Alzheimer's dementia (Smith, 1988). The nbm in rodents is considered the analogue of the nucleus basalis of Meynert in humans (Shaughnessy et al., 1996). This nucleus, which provides the major cholinergic input to the neocortex, undergoes massive cell loss in patients suffering from Alzheimer's disease. As a consequence, a large reduction in the activity of choline acetyltransferase, the enzyme that synthesizes acetylcholine (ACh), has been found in the neocortex of Alzheimer patients. The reduction of cortical presynaptic markers in the neocortex appears to be correlated with the severity of the dementia (Perry et al., 1978; Bierer et al., 1995).

Alzheimer's disease has also been found to be associated with a decline in sensorimotor abilities (Franssen et al., 1991; Kluger et al., 1997). A similar, although less pronounced, decline occurs in normal aging (Era, Jokela & Heikkinen, 1986), and an age-related decline in sensorimotor functions

has consistently been found in rodents (e.g. Marshall, 1982; Gage et al., 1983; Schuurman et al., 1986; Ingram, 1988; Markowska et al., 1990).

It is conceivable that lesioning of the nbm in aged rodents could provide a model of Alzheimer's disease that shares more aspects of the Alzheimer symptomatology than lesioning of the nbm of young rodents would do, because aging *per se* appears to lead to degenerative changes in the basal forebrain nuclei of the rat (Fischer, Gage & Björklund, 1989). These naturally occurring changes may make the aged rat brain more vulnerable to the effects of excitotoxin-induced nbm lesions (Zawia, Arendash & Wecker, 1992; Stoehr & Wenk, 1995). In order to test this hypothesis and to assess the effects of aging, of ibotenic acid induced lesions, and of the interaction between age and lesion, we tested young and aged Wistar rats in a battery of behavioral tests that have previously been found to be sensitive to aging or to the lesioning of particular brain regions. Learning and memory were assessed in a seven-choice discrimination task in a radial alley maze, which has been found to be sensitive to the effects of aging (see Appendix 6.2, and Raaijmakers et al., 1990) and to the effects of lesioning the nbm in rats (see Chapter 3.1).

We used a battery of functional tests for different reasons. First, in rodent aging studies it has been demonstrated repeatedly that the age-related decline in sensorimotor functions and in cognitive behavior is not a homogeneous process but can be differentiated on the basis of individual processes (Ingram, 1983; van der Staay, Blokland & Raaijmakers, 1990, and Chapter 2.3), i.e. age-related impairments might occur at different ages, depending on the behavior assessed.

Second, the nbm consists of magnocellular neurons which are diffusely distributed adjacent to, and perhaps within, the substantia innominata, the ventral globus pallidus, and the dorsal part of the dorsal globus pallidus (Everitt et al., 1987). The globus pallidus is a brain region which appears to play an important role in the regulation of movements (Parent & Hazrati, 1995a,b). Lesion-induced sensorimotor impairments might interfere with the interpretation of possible disruptive effects on learning and memory, as assessed in cognitive tasks such as the seven-choice task.

Third, functional tasks appear to be less affected by learning processes. They might, therefore, provide unbiased information about the disruptive effects of bilateral lesioning of the nbm and functional recovery (see also van der Staay, Augstein & Horváth, 1996a; but see Ingram & Reynolds, 1986, who found that certain psychomotor tests might also be confounded by learning).

Finally, because the location and size of the lesioned area might extend to brain regions other than the nbm, the use of different tests makes it more likely that lesion-induced deficits will be detected.

We assessed the effects of bilateral lesions on the performance in a spatial discrimination task because spatial discrimination performance has been found to be impaired in elderly humans (Light & Zelinski, 1983; Evans et al., 1984; Bruce & Herman, 1986; Utzl & Graf, 1993), and more severely so in Alzheimer's patients (e.g. Liu, Gauthier & Gauthier, 1991). Spatial orientation tasks in rodents have been found to be sensitive to aging (e.g. Barnes, 1988a; van der Staay, van Nies & Raaijmakers, 1990; van der Staay & de Jonge, 1993), and this is also true for the seven choice discrimination task used in the present study (Raaijmakers et al., 1990; Appendix 6.2).

Material and Methods

Animals

Eighteen 4-month-old and nineteen 27-month-old male Wistar rats (Cpb:Wu) were supplied by CPB, Zeist, the Netherlands. The aged rats had been transferred to Organon International b.v., Oss, the Netherlands at the age of 24 months, where they were kept in the animal house until they were moved to the animal facilities of the Psychological Laboratory, University of Nijmegen, the Netherlands, at the age of 27 months. These rats weighed between 412 to 516 grams. The young animals were moved directly from the CPB to the Psychological Laboratory. The young rats weighed between 286 and 372 grams.

Table 1. Protocol of the experiment, in which the effects of bilateral lesions of the nucleus basalis magnocellularis (nbm) on the sensorimotor function and performance in a seven-choice task in an eight-arm radial alley maze of young and aged Wistar rats were assessed. The exact week numbers (*: after arrival at our laboratory), the approximate ages in months, and the treatments and testing procedures are listed. **: Note that the young rats were 7 months old during the last testing series, an age at which they can be considered adult. However, throughout this paper, the group is referred to as 'young' or 'young adult'.

Week*	Approximate age (months)		Event
	young**	old	
0	4	27	Arrival at our laboratory, individual housing Reversed day/night cycle (lights on from 20:00 to 08:00)
1-2	4.5	27.5	Handling, open-field tests (data not shown) Pre-lesion functional examination
3			Bilateral lesioning of the nbm or sham operation
5	5	28	First post-lesion functional examination
6			Adaptation sessions in the radial alley maze
7-9	6	29	Acquisition of seven-choice task
10-11			Acquisition of a three-choice task (exploratory, data not shown)
12	7	30	Second post-lesion functional examination Retention of seven-choice task
13			Removal of the brains for histology and biochemical assays

All animals were housed individually in standard Makrolon[®] type III cages in a humidity and temperature controlled vivarium. They were habituated to a reversed day/night cycle (lights on from 20:00 to 08:00). The animals received food (standard rodent chow, Hope Farms) and water ad libitum. The experimental protocol is summarized in Table 1.

Nine 4-month-old and nine 28-month-old rats were randomly assigned to groups which received bilateral ibotenic acid lesions of the nbm in the course of the study. All other rats received bilateral sham lesions of the nbm.

Surgical procedure

The animals received stereotaxically guided lesions in the nbm, using a stereotaxic instrument (David Kopf). The rats were anesthetized with a combination of Vetalar® (i.m.: 50 mg/kg) and Rompun® (s.c.: 2.25 mg/kg) (Guldin & Markowitsch, 1981, 1982). The bilateral nbm lesions were produced by slow injection of ibotenic acid (4 µg in 0.4 µl phosphate buffer: pH 7.4) over 3.5 minutes at the coordinates: AP -0.8, L 2.7, and DV 8.0 (with respect to bregma and to the surface of the skull; Paxinos & Watson, 1986). The tip of the Hamilton syringe was kept in place for 5 extra minutes and then the syringe was retracted. The sham-lesioned rats received the same treatment as the nbm-lesioned rats, but were given injections of phosphate buffer (0.4 µl, pH 7.4).

In the first 3 to 4 hours after surgery, the ibotenic-acid treated rats had seizures, with profound arching of the back. From previous studies we knew that during this period, the animals gnawed continuously, mostly their own forepaws (O'Connell, Earley & Leonard, 1994). To prevent this automutilation, we fitted the animals with a ruff which they wore for the first 8 hours after surgery (see Chapter 3.1, Fig. 1).

Functional examination

About 1 week before the animals were operated on, baseline sensorimotor function was evaluated in a series of behavioral tests. The test battery consisted of a square bridge task, two grid tasks, two placing tasks, and a swim task. The first post-lesion functional examination was performed approximately 1 week after the operation. The second post-lesion functional examination was performed 9 weeks after lesioning.

Traversing a square bridge: a rat was placed on a square bridge that was about 50 cm above the surface, equidistant from two escape platforms (Wallace, Krauter & Campbell, 1980b). The bridge was 2 cm wide for the young adult rats and 6 cm wide for the aged rats. The diameter of the platforms was 12.5 cm for the young adult and 17.5 cm for the aged rats. Preliminary results (data not shown) indicated that aged rats of the strain used were unable to stay on the bridge unless its width was increased to 6 cm.

A rat was taken from its homecage and placed on one of the escape platforms for 15 seconds. Then, the rat was gently lifted by its tail and released as soon as it held the bridge with its four paws. The time the rat stayed on the bridge was measured to a maximum of 60 seconds. When a rat escaped to one of the platforms, the time was ascribed the maximum. A thick layer of plastic foam was placed beneath the bridge to cushion the rat's fall if it fell off. During the task, the room was illuminated by white strip lights. This test was performed twice in close succession: the rat was released in the middle of the bridge with its nose pointing to the left platform, and a second time with its nose pointing to the right escape platform. The mean of the two fall-off latencies was taken as score.

The next two tests were performed using a grid (75 cm width x 100 cm height) made of a stainless steel bars (diameter 5 mm). The grid consisted of a 13 x 17 matrix of 5 x 5 cm holes. One short side of the grid was attached to a stainless steel table. The other side was attached to the ceiling by nylon threads. The experimenter could manipulate the incline of the grid by pulling the nylon threads. A mirror was positioned under the grid to allow recording of the movement of the rat's feet by video equipment, in order to facilitate reliable scoring of paw placement. In order to reduce distracting visual stimuli, the room was dimly illuminated by red strip lights.

Turning on the inclined grid was assessed by a modification of the procedures described by Marshall (1982) and by Whishaw, Connor, and Dunnett (1985). The grid was held in a horizontal position. A rat was placed on it, approximately in the center. The nose of the rat pointed to the edge of the grid that

was to be lowered. Then, the grid was lowered until it attained a negative inclination of 30° with respect to the horizontal plane. The latency to turn on the grid was measured to a maximum of 60 seconds. If a rat turned 90° or more within 1 minute of lowering of the grid, a turning score of 1 was given. If the rat failed to turn on the grid, a turning score of 0 was awarded and the latency was set to 60 seconds. This test was performed twice in close succession. The mean of the two turning latencies was taken as the score. In addition, the sum of the two turning-scores was analyzed. The turning-score could range from 0 (rat never turned on the grid) to 2 (i.e. the rat turned on the grid in both trials).

Climbing on the inclined grid was assessed by a modification of the procedures described by Marshall (1982) and by Whishaw, O'Connor, and Dunnett (1985). Observation started immediately after a rat had turned 180°. Rats which failed to turn were turned by the experimenter. A step was operationally defined as the movement of a paw from one side of a square to one of the three other sides of the same square or to one of the sides of the adjacent squares. The number of steps was counted. Steps were classified as correct whenever a paw was placed on the grid. A step was classified as incorrect (misstep) whenever the rat put a paw through one of the holes, irrespective of whether or not the rat corrected this step. When the rat had made at least seven steps, it was taken from the grid and placed in its homecage. Scoring of climbing on the grid was done from the videotapes. The means of the scores of the two successive trials were analyzed.

Visually triggered placing: the test was adapted from Marshall (1982). A rat was picked up by its tail and was slowly lowered toward the edge of a table until its nose was approximately 10 cm from the edge. The white tabletop contrasted sharply against the dark floor. Care was taken that the vibrissae did not touch the edge. A placing-score of 1 was awarded when a rat extended its forepaws towards the edge (the rat showed visual placing). Otherwise, a placing score of 0 was given. The sum of the two placing scores was analyzed. This score could range from 0 (rat never showed visually triggered placing) to 2 (i.e. the rat showed visually triggered placing in both trials).

Contact placing: this task resembles *visually triggered placing*. The rat was lowered until its vibrissae touched the edge of the table (Whishaw, O'Connor & Dunnett, 1985). If a rat extended the forelimbs toward the edge as soon as it had made tactile contact with the table with its vibrissae, a placing score 1 was given. Otherwise, a placing score of 0 was given. The sum of the two placing-scores was analyzed. This score could range from 0 (rat never showed contact placing) to 2 (i.e. the rat showed contact placing in both trials).

Swim task: swimming behavior was assessed by a modification of the procedure described by Marshall and Berrios (1979). A glass aquarium (length: 80 cm, depth: 50, width: 40 cm) was used, filled to a depth of 35 cm with water that was kept at 30°C. The behavior of the animals was videotaped for a maximum of 5 minutes. The experimental room was illuminated by white strip lights. Swimming was scored by using two rating scales, one for swimming vigor and the other for swimming success (Marshall & Berrios, 1979).

Swimming vigor was rated on a scale ranging from 3 to 0. A score of 3 was awarded when the rat moved its four limbs continuously. The score 2.5 was given when the rat occasionally floated, whereas floating more than swimming was assigned the value 2. When the rat only occasionally swam using its four limbs, a 1.5 was given. Occasional swimming using the hindlimbs only was assigned the value 1. A rat that did not use its limbs received the score 0.

Swimming success was awarded the maximum value of 3 when the rat was able to keep its entire head above the water. When the ears, but not the eyes were under water, a score of 2.5 was given. A

2 was awarded when the rat usually had its eyes, but not its nose under water. When the head disappeared under the surface of the water for 6 seconds or longer the score 1 was awarded. A rat that sank to the bottom of the aquarium for 10 seconds or longer was given the score 0. If a rat scored 1 or 0 on swimming success for the first time, it was immediately taken out of the water tank and observation was discontinued.

Statistical analysis

The effects of age and lesions on the performance of the rats across the three testing sessions (one pre-lesion, and two post-lesions assessments) were analyzed by analysis of variance (ANOVA) with the factors Age (young vs. old), lesion (sham-lesion vs. nbm-lesion) and the repeated measures factor Sessions (pre-lesion session vs. first and second post-lesion session). The same analyses were also performed on the ranked scores, because for some of the measures floor or ceiling effects were seen in particular groups. These effects might violate the assumptions underlying ANOVA. In addition, non-parametric Kruskal-Wallis analyses were performed per session, with the four Age by Lesion groups as levels of Group. However, the results of the three statistical approaches were virtually identical and in no case gave rise to different conclusions. Therefore, the results of the repeated measures analyses will be reported, and data are depicted graphically as means and standard errors of the means.

Swimming success and swimming vigor were analyzed by ANOVA with the factors Age (young vs. old), lesion (sham-lesion vs. nbm-lesion) and the repeated measures factors Sessions (pre-lesion session vs. first and second post-lesion session) and Blocks (minute 1 to 5 of a session).

Seven-choice task

Apparatus

The eight-arm radial alley maze consisted of a central platform (diameter: 26 cm) from which eight alleys radiated equidistantly (see Chapter 3.2, Fig. 1). The alleys measured 46 cm in length and 10 cm in width. The gray side walls were 38 cm high. All walls were made of gray polyvinyl chloride, except the end walls of the alleys, which were made of aluminum. A recess (5.0 cm * 4.0 cm * 4.7 cm) that contained a food tray was built about 2 cm above the floor into the end walls. The floor of the whole apparatus was made of Trespa®.

A cylindrical door (diameter: 49.5 cm, 16.6 cm above the floor when closed) that opened by moving down vertically until its upper edge reached the level of the floor of the apparatus allowed simultaneous access to the eight alleys. Hidden behind the closed door, about 5 cm above the floor of each alley, were black symbols on white, illuminated screens measuring 9.5 * 9.5 cm (see Chapter 3.2, Fig. 2). When the door was open, the symbols provided distinct intra-maze cues in each alley.

The experimenter opened and closed the door. Visits to the alleys (operationalized as entering the alley by a rat's body length) and to the food cups were registered automatically by photosensitive cells. An Apple IIe microcomputer collected the data and controlled the duration of each trial. The radial alley maze was situated in an experimental room that was illuminated by four red fluorescent strip lights and three 100-W bulbs, which were adjusted by a dimmer to provide illumination of about 50 lux on the floor of the apparatus.

The room had three doors, two one-way screens, and one window (screens and window covered with black curtains). Further, the room contained a sink, a table along one wall on which the computer and the interface were situated, and a holeboard apparatus. None of these potential extra-maze cues,

however, was visible to the rat in the alley maze. The experimenter sat in a chair in front of the maze, and was not visible to the rat during testing.

Procedure

Starting 2 weeks after surgery, the body weights of all young animals were gradually reduced to 85%, whereas those of the aged rats were gradually reduced to 77.5% of their free-feeding values. This differential deprivation schedule was applied in order to reduce the difference in motivation between the young and aged rats (Blokland & Raaijmakers, 1993b). When the rats had reached their target weights, growth correction was applied for the young rats during the period of deprivation, using the growth curves of non-deprived male Wistar peers. All rats were kept on the food deprivation schedule for 6 weeks, i.e. until retention had been tested.

Adaptation: during the first deprivation week, the rats were familiarized with the radial alley maze on 4 consecutive days (10 min/day). These adaptation sessions started 24 hours after the rats had been put on the restricted feeding regimen. The rats were put in one alley (no. 1) and then the circular door was opened, allowing free access to all parts of the apparatus. The food trays of every alley, except that of the start alley, were baited with one food pellet (45 mg, Campden Instruments). Additional pellets were scattered on the floor of the alleys and of the central platform. The start alley was distinct from the other seven alleys: at the entrance to the center of the apparatus the rats had to cross a low barrier (1.5 cm high).

Acquisition: when spatial discrimination learning in the seven choice task began (1 week after the start of the adaptation sessions), all rats had reached their respective target weight. During training the rats were started from alley no. 1. The alley 135° to the right of the start alley (no. 6) contained the food reward (four 45-mg food pellets). A trial was terminated as soon as the rat had found the food or when 10 minutes had elapsed, whichever event occurred first. Rats could enter and re-enter all alleys (including the start alley) freely during a trial. Thus, a correction procedure was applied. All rats were trained with massed trials (days 1-3: 2 trials/day; days 3-10: 3 trials/day; days 11-end of training: 6 trials/day), until they had reached a criterion of seven faultless trials in a series of nine trials.

Retention: retention of the seven-choice task was assessed 6 weeks after the start of acquisition. Again, the rats were trained in massed trials (6 trials/day). Most rats reached criterion within three sessions. Very few rats needed a fourth session, in which the number of trials was increased to nine.

Statistical analysis

One measure of the adaptation sessions was analyzed statistically: number of alley visits.

- *Number of alley visits* is the total number of entries into the alleys of the maze during a 10-minute adaptation session. This measure comprises information about exploration and the speed of adaptation (changes in the number of alleys visited over adaptation sessions).

The effects of age and lesion on the adaptation sessions were analyzed by an Age (young vs. old) by Lesion (sham-lesioned vs. nbm-lesioned) by Sessions (sessions 1 to 4) ANOVA with repeated measures on the last factor.

Two measures of the acquisition and the retention training were analyzed statistically: trials to criterion and errors to criterion.

- Trials to criterion were the number of trials needed to reach the criterion of seven faultless trials in a series of nine trials. The criterion trials are included.

- Errors to criterion were the number of errors (visits and re-visits of the never baited alleys, including all visits to the start alley) summed over all trials to criterion.

The effects of age and lesions on the two measures, trials and errors to criterion, were analyzed by a Age (young vs. aged rats) by Lesion (sham-lesioned vs. nbm-lesioned) ANOVA.

Dissection of brain samples

Brain samples were dissected 10 weeks after lesioning. The rats were decapitated without anesthesia, and the severed head was kept in liquid nitrogen for about 5 seconds to cool the brain. Then, the brain was rapidly removed and dissected at 4-10°C in an open refrigerator.

Frontal and parietal cortex samples were dissected. In the coronal plane the cortical sample was delimited with a calibrated plastic T-square (Rosenzweig, Bennett & Diamond, 1972; Raaijmakers 1978; see also Chapter 3.2, Fig. 3); the tissue under the area covered by the T-square was discarded. Rostrally, the frontal cortex sample was delimited by a horizontal knife-cut above the olfactory bulbi. In addition, the dorsal hippocampus was taken from a subset of the animals (see Table 2).

Table 2. The number of hippocampal and cortical samples analyzed from sham-lesioned and nucleus basalis magnocellularis (nbm)-lesioned young and old Wistar (WU:Cpb) rats.

Brain sample	Young rats		Old rats	
	sham-operated	nbm-lesioned	sham lesioned	nbm-lesioned
Frontal cortex	9	9	10	9
Parietal cortex	9	9	10	9
Dorsal hippocampus	6	6	4	4

Choline acetyltransferase (ChAT) in the brain samples

The ChAT activity in the different brain samples was measured by the methods described in Chapter 3.1.

Histological verification of lesions

Care was taken not to damage the brain tissue underlying the cortex and hippocampus, respectively. This part of the brain was stored in 4% formalin solution for histological verification of the nbm lesions. The size and location of the nbm lesions were assessed in coronal sections (40-µm thick) cut through the entire lesioned area, using a cryostat microtome. The coronal sections chosen for histological verification roughly corresponded to the levels -0.3, -0.8, -1.3, and -1.8 mm from bregma in the stereotaxic atlas of Paxinos and Watson (1986). Slide-mounted tissue sections were stained with cresyl fast violet and examined microscopically.

Results

One sham-lesioned and three nbm-lesioned aged rats died during the 2½-month post-surgery period in which behavioral testing was performed. Consequently, the data for these animals were not considered

in the statistical analyses and the results reported are from 9 sham-lesioned young rats, 9 nbm-lesioned young rats, 10 sham-lesioned old rats, and 9 nbm-lesioned old rats. Unfortunately, data were lost during the functional examinations in the sensorimotor testing battery. Therefore, some analyses were based on data from fewer animals.

Behavioral testing

Functional examinations

Traversing a square bridge: latency to fall off (see Fig. 1, left panel): repeated measures analysis across the three sessions revealed that, on average, the aged rats had a shorter fall-off latency than the young rats (General mean: $F_{1,33} = 25.33$, $p < 0.01$). Evaluation of the pre-lesion performance revealed that the latency to fall off the bridge was shorter for the aged rats than the for young rats from the first testing session onward (Age: $F_{1,33} = 12.25$, $p < 0.01$, Lesion: $F_{1,33} = 3.42$, $0.1 > p > 0.05$; Age by Lesion interaction: $F_{1,33} < 1.0$, n.s.). However, the latencies did not change over sessions ($F_{2,66} = 1.91$, n.s.), nor were there any differential effects of the nbm lesion on the performance of the two age groups (all interactions between Age, Lesion and Sessions: $F_{2,66} < 1.0$, n.s.).

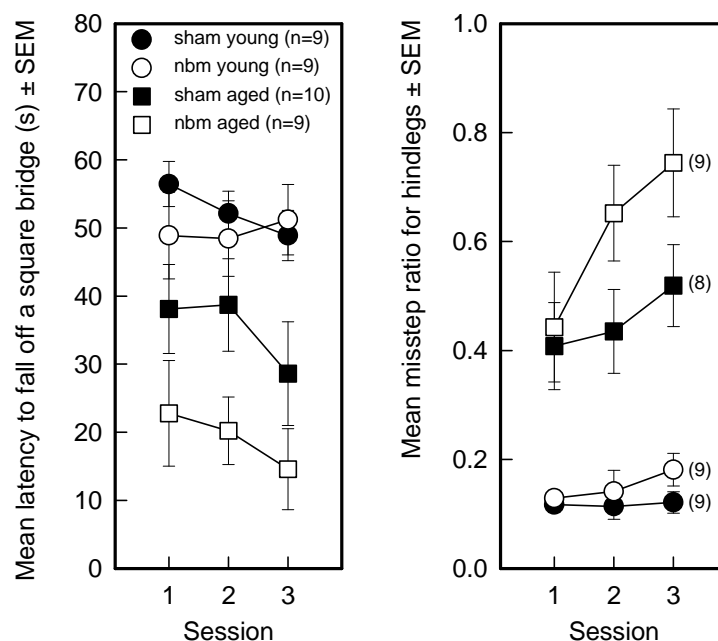


Figure 1. Latency to fall off a square bridge (left panel) and misstep ratio for hindlegs (right panel) of sham-lesioned and nucleus basalis magnocellularis (nbm)-lesioned young and old Wistar rats. The means and standard errors of the means (SEM) are depicted. Due to missing values, the number of sham-lesioned aged rats in the right panel was reduced to eight (number of animals are shown between parentheses).

Turning on the inclined grid (data not shown): owing to missing values, only the data of 36 rats were analyzed. The average number of turns on the inclined grid was higher for the young rats than for the old animals (General mean: $F_{1,32} = 14.29$, $p < 0.01$). Lesioning of the nbm appeared to affect the average number of turns on the grid differently (Lesion: $F_{1,32} = 1.21$, n.s., Age by Lesion interaction: $F_{1,32} = 6.61$, $p < 0.05$). Fisher LSD post-hoc comparison revealed that the lesion effect was due to a difference between the sham- and the nbm-lesioned old rats. The nbm-lesioned old rats made fewer turns than their sham-lesioned peers. Evaluation of the pre-lesion performance on this task revealed that this difference was already observable before lesioning (Age: $F_{1,32} = 4.50$, $p < 0.05$; Lesion: $F_{1,32} < 1.0$, n.s.; Age by Lesion interaction: $F_{1,32} = 8.08$, $p < 0.01$).

Over Sessions, the number of turns remained stable and was not affected by the lesions (Sessions: $F_{2,64} = 1.29$, n.s.; all interactions between Age, Lesion and Sessions: $F_{2,64} < 1.0$, n.s.). The difference observed between the sham- and the nbm-lesioned groups of old rats, therefore, appears to be the result of the random group assignment.

Climbing on the inclined grid: missteps with forelegs (data not shown): the number of missteps on the inclined grid with the forelegs was not affected by age or by lesioning of the nbm.

Climbing on the inclined grid: missteps with hindlegs (see Fig. 1, right panel): owing to missing values, only the data of 35 rats were analyzed. A different picture occurred for number of missteps with hindlegs: the aged rats made, on average, more missteps than the young rats (General mean: $F_{1,31} = 45.68$, $p < 0.01$).

Over sessions, the number of missteps with the hindlegs increased (Sessions: $F_{2,62} = 9.58$, $p < 0.01$), differently for the two age groups (Age by Sessions interaction: $F_{2,62} = 5.73$, $p < 0.01$). The increase was more pronounced for the aged rats than for the young rats. Lesioning of the nbm did not affect the number of missteps (Lesion by Session interaction: $F_{2,62} = 2.27$, n.s.; Age by Lesion by Session interaction: $F_{2,62} = 1.22$, n.s.).

Visually triggered placing (data not shown): the aged rats showed, on average, less visually triggered placing than the young rats did (General mean: $F_{1,33} = 8.11$, $p < 0.01$). Visually triggered placing decreased across the three testing sessions ($F_{2,66} = 19.36$, $p < 0.01$), but was not affected by age or by lesioning of the nbm.

Contact placing (data not shown): contact placing could be elicited in all rats in each testing session.

Swim task (data not shown)

Swimming vigor: owing to missing values, the data of 36 animals were analyzed. On average, the young rats swam more vigorously than the aged rats (General mean: $F_{1,32} = 9.71$, $p < 0.01$). Swimming vigor decreased differently over the three testing sessions (Sessions: $F_{2,64} < 1.0$; Age by Sessions interaction: $F_{2,64} = 3.20$, $p < 0.05$), but this decrease appeared to be restricted to the aged rats. Lesioning of the nbm did not affect the swimming vigor over testing sessions (Lesion by Sessions interaction: $F_{2,64} = 2.25$, n.s.; Age by Lesion by Sessions interaction: $F_{2,64} < 1.0$, n.s.). Within sessions, swimming vigor decreased over the 5 minutes (Blocks: $F_{4,128} = 26.27$, $p < 0.01$). The decrease within sessions was stronger for the aged than the young rats (Age by Blocks interaction: $F_{4,128} = 3.36$, $p < 0.05$). This decrease was not affected by lesioning of the nbm (both interaction terms: $F_{4,128} < 1.0$, n.s.), nor was the within-sessions decrease different across the three testing sessions (all $F_{8,256} < 1.0$, n.s.).

Swimming success: on average, the swimming success of the young rats was superior to that of the aged rats (General mean: $F_{1,32} = 4.20$, $p < 0.05$). Swimming success did not change over sessions ($F_{2,64} \leq 1.09$, n.s., for Sessions and all interactions of Age, and Lesions with Sessions). Within sessions, swimming success decreased over the five 1-minute blocks ($F_{4,128} = 23.25$, $p < 0.01$). Neither age nor lesions affected the decrease in swimming success within sessions (all $F_{4,128} < 1.0$, n.s.). The within-sessions decrease was also not different across the three testing sessions (all $F_{8,256} < 1.0$, n.s.).

Seven-choice task: adaptation sessions

Number of alley visits (see Fig. 2, left panel): averaged over the four adaptation sessions, the aged rats made fewer alley visits than the young rats did (General mean, Age: $F_{1,33} = 91.25$, $p < 0.01$). Lesioning

had no effect on the mean number of alley visits (Lesion: $F_{1,33} = 2.47$, n.s., Age by Lesion interaction: $F_{1,33} = 2.47$, n.s.).

The number of alley visits was stable across the four adaptation sessions (Sessions: $F_{3,99} < 1.0$, n.s.), and this was true for the two age groups (Age by Sessions interaction: $F_{3,99} = 1.21$, n.s.) and the lesion conditions (Lesion by Sessions interaction: $F_{3,99} < 1.0$, n.s.; Age by Lesion by Sessions interaction: $F_{3,99} < 1.0$, n.s.).

Acquisition of the seven-choice task

Trials to criterion (see Fig. 2, center panel): the young animals needed fewer trials to reach the criterion than the aged rats did (Age: $F_{1,33} = 16.15$, $p < 0.01$). Lesioning of the nbm did not affect the number of trials needed to reach criterion (Lesion: $F_{1,33} < 1.0$, n.s.; Age by Lesion interaction: $F_{1,33} < 1.0$, n.s.).

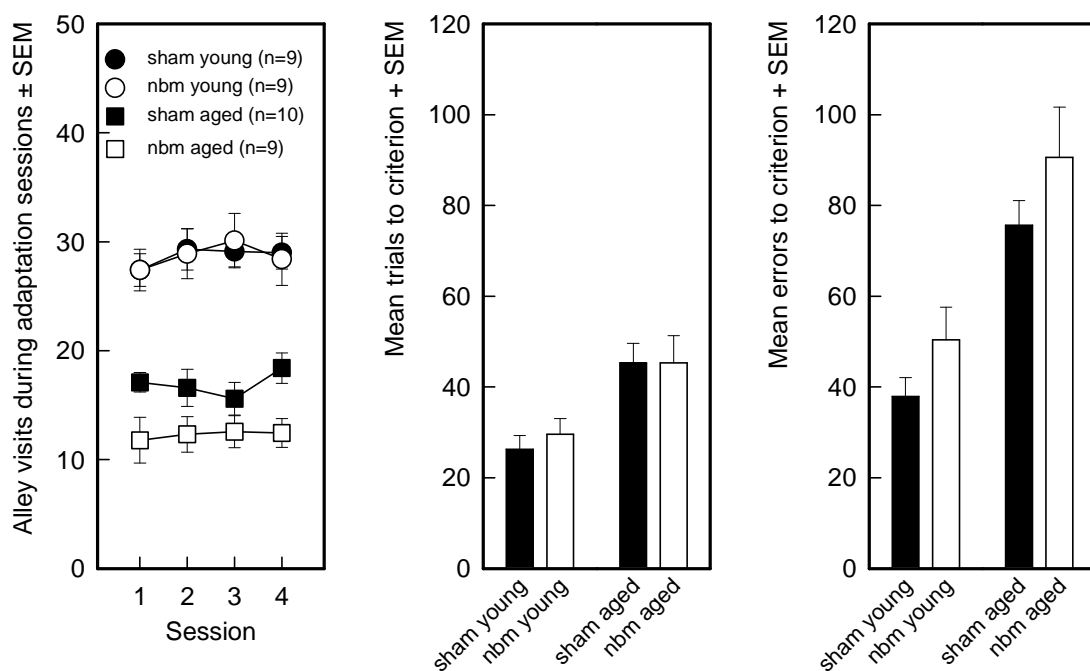


Figure 2. Mean number of alley visits \pm standard errors of the means (SEM) during the adaptation sessions (left panel), trials to criterion (center panel) and errors to criterion (right panel) during acquisition of a seven-choice task in a radial alley maze of sham-lesioned and nucleus basalis magnocellularis (nbm)-lesioned young and old Wistar rats.

Errors to criterion (see Fig. 2, right panel): the young rats made fewer errors than the old rats to reach criterion (Age: $F_{1,33} = 28.04$, $p < 0.01$). The nbm-lesioned animals made more errors than the sham-lesioned rats (Lesion: $F_{1,33} = 3.50$, $p < 0.05$); the magnitude of this effect was similar in both age groups (Age by Lesion interaction: $F_{1,33} < 1.0$, n.s.).

Retention of the seven choice task

Trials to criterion (see Fig. 3, left panel): the number of trials to criterion was not affected by age ($F_{1,33} = 2.66$, n.s.) or by the nbm lesion ($F_{1,33} = 1.15$, n.s.; Age by Lesion interaction: $F_{1,33} = 1.04$, n.s.).

Errors to criterion (see Fig. 3, right panel): the young rats tended to make fewer errors than the old rats did to reach criterion during retention testing (Age: $F_{1,33} = 3.38$, $0.10 > p > 0.05$). In this phase of the experiment, the nbm lesions did not affect the number of errors (Lesion: $F_{1,33} = 64$, n.s.; Age by Lesion interaction: $F_{1,33} = 1.31$, n.s.).

ChAT activity in frontal and parietal cortices and in hippocampus

Frontal cortex: ChAT activity in the frontal cortex was not different in the two age groups ($F_{1,33} < 1.0$, n.s.; see Fig. 4. left panel). The nbm lesion reduced the ChAT activity in the frontal cortex by about 25% ($F_{1,33} = 38.62$, $p < 0.01$) when compared with that of the sham-lesioned rats. The decrease was marginally different for the two age groups (Age by Lesion interaction: $F_{1,33} = 3.86$, $0.10 > p > 0.05$). The effect of the lesion appeared to be more pronounced in the aged rats than in the young rats.

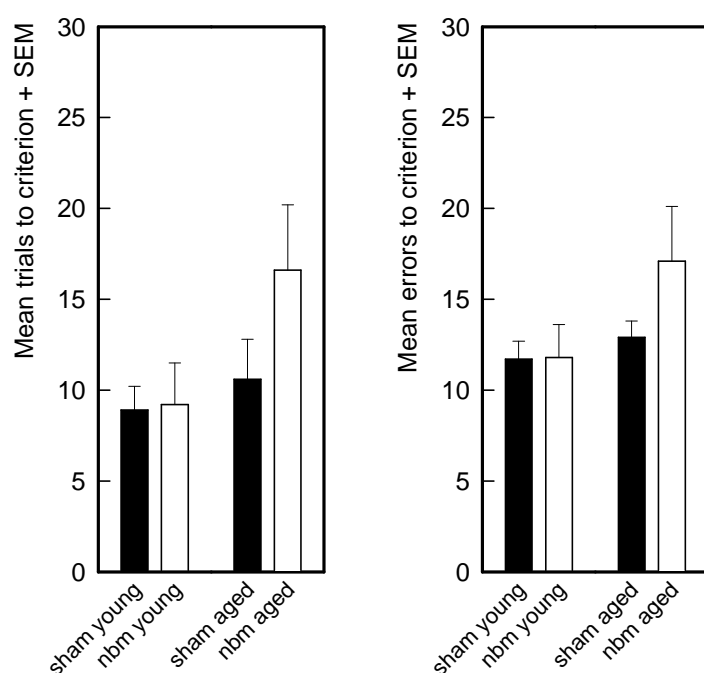


Figure 3. Trials to criterion (left panel) and errors to criterion (right panel) of sham-lesioned and nucleus basalis magnocellularis (nbm)-lesioned young and old Wistar rats in seven-choice task in a radial alley maze. The means and the standard errors of the means (SEM) of the performance during the retention are depicted.

Parietal cortex: as for the frontal cortex, ChAT activity in the parietal cortex was not different in the two age groups ($F_{1,33} < 1.0$, n.s.; see Fig 4. right panel). The nbm lesion reduced the ChAT activity in the parietal cortex by about 16% ($F_{1,33} = 24.96$, $p < 0.01$). There were no age differences or Age by Lesion interactions on this measure ($F_{1,33} = 2.16$, n.s.).

Hippocampus: ChAT activity in the dorsal hippocampus was not affected by age or by lesioning of the nbm (all $F_{s,16} < 1.0$, n.s.).

Histological verification of the nbm lesions

The size and location of the lesions were in good agreement with those seen in the previous experiments. Schematic representations of lesions induced by injections of 0.4 μ l ibotenic acid at the coordinates AP -0.8, L 2.7, and DV 8.0, with respect to bregma and to the surface of the skull (Paxinos

& Watson, 1986), are depicted in 3.2, Fig. 9, right panel. There were no differences between the two age groups.

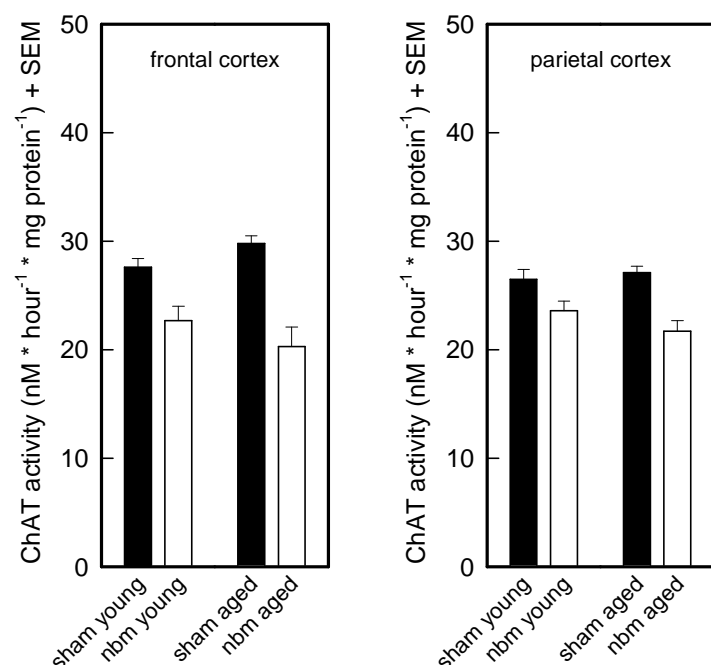


Figure 4. Choline acetyltransferase (ChAT) activity in the frontal cortex (left panel) and the parietal cortex (right panel) of sham-lesioned and nucleus basalis magnocellularis (nbm)-lesioned young and old Wistar rats. The means and the standard errors of the means (SEM) are depicted.

Discussion

In the present study, we investigated the effects of aging, bilateral lesioning of the nbm, and possible interactions between these two factors. The results are summarized in Table 3. We hypothesized that the effects of nbm lesions would be more severe in aged rats than in young rats, because aging *per se* has been found to affect rat's behavior, ranging from sensorimotor impairments (e.g. Marshall, 1982; Gage et al., 1983; Gage, Dunnett & Björklund, 1984; Ingram, 1988; Markowska et al., 1990) to learning and memory deficits (e.g. Gage, Dunnett & Björklund, 1984; Rapp & Gallagher, 1996; van der Staay, Hinz & Schmidt, 1996a).

The study confirmed that aging affected the behavior of Wistar rats in the functional test battery and in the spatial learning task. Compared with the clear age-associated behavioral impairments found, the effects of bilateral lesioning of the nbm on the behavior of rats in the two age groups were, at best, moderate. They appeared to be entirely restricted to the learning phase of the seven-choice task. The lesions did not affect performance of the functional tests, corroborating the findings of others (e.g. Flicker et al., 1983; Wozniak et al., 1989; O'Connell, Earley & Leonard, 1994). The lesions, as verified histologically and biochemically, were relatively small, i.e. did not extend into adjacent structures, and their size and position were comparable with those of lesions in the nbm produced by others (e.g. Everitt et al., 1987; Shaughnessy et al., 1996).

Table 3. Summary of the results of the behavioral testing of nine sham-operated young, nine nucleus basalis magnocellularis (nbm)-lesioned young, ten sham-operated old and nine nbm-lesioned old Wistar rats.

*: Result most probably is an artifact of the random assignment of the rats to the four experimental groups. This difference between old, sham-lesioned, and nbm-lesioned rats already existed in the functional tests before the operation.

Test	Short description of effects	
	Age differences	Effects of nbm lesions
Functional examination in different tests		
1 Square bridge: fall-off latency	Old rats had shorter latencies than young rats	No effects of nbm lesion
2 Turning of grid: number of turns	Old rats turned less often than young rats did	Old nbm-lesioned rats turned less often than shams did*
3 Climbing on inclined grid: missteps with forelegs	No age differences	
4 Climbing on inclined grid: missteps with hindlegs	Old rats made more missteps than young rats	No effects of nbm lesion
5 Placing task: visually triggered placing	Old rats showed less visually triggered placing	No effects of nbm lesion
6 Placing task: placing triggered by contact	No age differences	No effects of nbm lesion
7 Swimming task: swimming vigor	Old rats swam less vigorously than young ones	No effects of nbm lesion
8 Swimming task: swimming success	Old rats swam less efficiently than the young ones	No effects of nbm lesion
9 Alley visits during adaptation in alley maze	Old rats made fewer alley visits than young rats	No effects of nbm lesion
Learning and memory in the seven-choice task		
10 Acquisition of seven-choice task: trials to criterion	Old rats needed more trials than young rats	No effects of nbm lesion
11 Acquisition of seven-choice task: errors to criterion	Old rats made more errors to reach criterion than young rats	Nbm-lesioned rats made more errors to criterion than shams
12 Retention of seven-choice task: trials to criterion	No age differences	No effects of nbm lesion
13 Retention of seven-choice task: errors to criterion	No age differences	No effects of nbm lesion
Choline acetyltransferase activity in brain samples		
14 Frontal cortex	No age differences	About 25 % reduction compared with shams
15 Parietal cortex	No age differences	About 16% reduction compared with shams
16 Dorsal hippocampus	No age differences	No effects of nbm lesion

Functional examination

During the adaptation phase preceding the seven-choice task, the aged rats were much less active than their young counterparts. In the bridge task, the aged rats had a shorter fall off latency than the young rats. They turned less on the inclined grid, made more missteps with their hindlegs when walking on the grid, and showed less visually triggered placing than the young animals did. The swimming behavior of the old rats was less vigorous and less efficient than that of the young ones. Within the 5-minute testing sessions of the swim task, the swimming vigor of the aged rats decreased faster than that of the young ones, whereas no such differential decrease during the test was seen for

swimming success. Aging did not affect the number of missteps with the forelegs during walking on the inclined grid or contact placing.

These findings corroborate results reported by others. In the square bridge test (e.g. Gage, Dunnett & Björklund, 1984; Schuurman & Traber, 1989b; Markowska et al., 1990), aged rats have been found to fall off much faster than young rats. Schuurman and colleagues (1986), who compared the motor coordination of rats of different ages (range: 3 to 24 months) on a round bridge (diameter 2.5 cm) and on square bridges 2.5 and 5 cm wide, found that there was a continuous, age-related decrease in the ability to stay on bridges 2.5 cm wide, with the round bridge being the more demanding bridge. This impairment already started in adult rats. Rats up to 18 months of age were able to stay on a 5-cm-wide bridge during the entire observation period, whereas 24-month-old rats could not. Marshall (1982) reported that visually triggered placing was impaired in aged rats, whereas contact placing was not. The effects of age on swimming success and swimming vigor in the swim task were not as pronounced as those reported by Marshall and Berrios (1979). Age effects on swimming behavior in a study by Gage, Dunnett and Björklund (1984), by contrast, were statistically confirmed for swimming success, whereas for swimming vigor only a trend toward an age-associated decline was seen.

The aged rats appeared to suffer from severe dysfunctions of their hindlegs, as has already been reported by Marshall (1982). These malfunctions were the main reason why we increased the width of the square bridge from 2 to 6 cm for the old rats. Even under these conditions, the fall-off latencies of the aged rats were shorter than those of the young ones. The age-associated impairments of the hindlimbs may have affected the behavior in other tasks, such as the turning and climbing on grid. In contrast to this strong age-related decline in hindlimb functions, the functional integrity of the forelimbs appears to be preserved up to a very old age in rats (e.g. Jürgens & Dinse, 1997). This may explain why there were no age differences in the placing tasks and on missteps with forelegs on the inclined grid, which depend, in addition to preserved visual and tactile abilities, on adequate use of the forelimbs.

Unfortunately, walking patterns were not assessed in the present study. Spengler and colleagues (1995) found a severe degradation of the functional representation in the somatosensory cortex in aged rats, the severity of which correlated with disturbances in hindlimb coordination, as assessed by footprint pattern analysis.

In age comparison studies older rats have been consistently found to make additional footsteps and slidings (Schuurman et al., 1987; Schuurman and Traber, 1989a,b; Gispen, Schuurman & Traber, 1988; van der Zee et al., 1989, Klapdor et al., 1997b). Dorner, Otte, and Platt (1996) found that these age-related alterations in the walking pattern of rats can be improved by muscle training. In our study, the extensive testing of the rats in the battery of functional tests and in the discrimination tasks did not improve the use of the hindlegs. Here, however, a strong age-related decrease in the physical condition of the rats might have interfered with putative performance-improving training effects.

Acquisition and retention of the seven-choice task in the radial alley maze

Our results were consistent with there being age-associated deficits of the acquisition of the seven-choice task (see Appendix 6.2, and Raaijmakers et al., 1990). The aged rats needed more trials and made more errors during the acquisition of the seven-choice task to reach the criterion of seven faultless trials in a series of nine trials. Once they had mastered this task, aged rats retained the correct route to the goal arm as well as the young rats, when retention performance in the seven-choice task was assessed 6 weeks after acquisition. There were, however, lasting age-related

impairments in the functional tests. These impairments, however, appeared to be unrelated to learning capacity, an observation that has also been made by others (e.g. Gallagher & Burwell, 1989).

A potential source for differences between age groups on appetitively motivated tasks has been signaled by Goodrick and co-workers (Goodrick, 1968; Ingram, London & Goodrick, 1981). When deprived to the same percentage of their free-feeding weights, older rats are less motivated than younger rats. In order to reduce the possibility that differences in motivational state are responsible for differences in performance between age groups, a differential deprivation technique was applied (van der Staay, van Nies & Raaijmakers, 1990; Blokland & Raaijmakers, 1993b): the senescent rats were deprived more than the young ones to induce a comparable level of motivation. Unfortunately, it cannot be completely ruled out that different levels of motivation were (partly) responsible for the age differences found in the seven-choice task, even when a differential deprivation technique was used.

The nbm lesion affected the acquisition of the seven-choice task by both age groups to a similar extent. The effect was transient and apparent for the measure 'errors to criterion', whereas no effect of the lesion was found on 'trials to criterion'. This indicates that the nbm-lesioned rats made more erroneous arm visits per trial during the *initial* phase of acquisition. If trials to criterion are considered, however, then these rats learned as quickly as their sham-operated peers. A similar transient effect of ibotenic acid-induced nbm lesions has been reported by Holley and co-workers (1993), using an operant visual conditional discrimination task, in which a particular stimulus, a flashing or a constant light, signaled whether responding to the left or right lever was reinforced. These results, however, contrast with the findings in the first experiment of Chapter 3.2, in which both the trials and errors to criterion were clearly affected by the nbm lesion in young Wistar rats.

No effects of the lesion were apparent when retention was tested 9 weeks after the operation. There are at least two alternative explanations for this finding.

First, this might have been due to recovery processes. Shaughnessy and colleagues (1996) trained rats with colchicine-induced nbm lesions or with sham lesions either 5 or 12 weeks after the operation in a standard Morris water escape task. They found that rats that acquired the platform escape response 5 weeks after the operation consistently performed poorer than the sham-lesioned rats. By contrast, sham- and nbm-lesioned rats acquired the task equally well when the acquisition sessions were run 12 weeks after the operation. These findings support the notion of functional recovery after nbm lesions.

Second, the lack of nbm lesion effects during retention testing 9 weeks after the acquisition is also congruent with the results of aging studies in which it was found that old rats retain spatial discrimination performance over very long retention intervals, i.e. months without further training (e.g. Beatty, Bierley, and Boyd, 1985; Bierley et al., 1986; Caprioli et al. 1991; van der Staay & Blokland, 1996b, and Chapter 2.3)

Because in the present experiment the animals acquired the task 5 to 6 weeks after the operations, and were re-tested approximately one month after acquisition, it remains unclear whether the first or the second explanation applies to our findings.

ChAT activity in the frontal and parietal cortices and in the hippocampus

There was no difference in ChAT activity in the three brain samples from the young and old rats. In laboratory rodents, the effects of aging on ChAT activity in the cortex and hippocampus appear to be highly variable, ranging from clear decreases to no age-related changes to clear increases in aged rats and mice (e.g. Decker, 1987; Sherman & Friedman, 1990). However, a significant number of these

studies did not find any age-related changes in ChAT activity. Interestingly, Sherman and Friedman (1990) found a reduction of sodium-dependent high-affinity choline uptake, a marker for cholinergic neuronal activity and structural identity, in aged C57/BL mice, whereas the activity of ChAT, a structural marker, was not affected by aging. Measurement of ChAT activity alone is thus inadequate as an indicator of the functional state of cholinergic systems.

Ten weeks after lesioning, the ChAT activity in the cortical samples from nbm-lesioned rats was clearly decreased. The nbm lesion reduced ChAT activity in the frontal cortex by about 25% and ChAT activity in the parietal cortex was reduced by about 16%, compared with that of the sham-lesioned groups. The decrease seemed to be more pronounced in the frontal cortex than in the parietal cortex, an observation that was consistent across experiments. Other authors have reported similar reductions in cortical ChAT activity after ibotenic acid lesions of the nbm (e.g. Dokla & Thal, 1989; Shaughnessy et al., 1994; Liljequist et al., 1997). No effects were seen on hippocampal ChAT activity.

The decrease in ChAT activity was similar in both age groups. This observation does not corroborate findings reported by Zawia, Arendash, and Wecker (1992) that aged rats are more susceptible to neuronal degeneration as a consequence of nbm lesions, or findings by Luiten and co-workers (1995) that aged rats are less susceptible than young rats to NMDA-induced damage of the nbm.

The decreases in ChAT activity in the frontal and parietal cortex samples, measured 10 weeks after the operation, were only moderate and might reflect the effects of recovery processes. An exploratory analysis of the data of the present experiment and of age-matched young and old Wistar rats, in which the ChAT activities were determined one week after lesioning, supports this notion. The ChAT activity in the frontal and parietal cortices of age-matched young and old Wistar rats was reduced to 47% and 35% of the activity measured in sham-lesioned controls, 1 week after nbm lesioning, compared with a decrease of 25% and 16% in the two cortical samples after a 10-week survival period (for details, see Appendix 6.4).

Wenk and Olton (1984) reported that neocortical ChAT activity recovered completely 3 months after unilateral lesioning of the nbm in Sprague Dawley rats, in contrast to a reduction of approximately 60%, 7 days after lesioning. They concluded that the basal forebrain cholinergic system shows enormous plasticity and that its function can completely recover. Partial and region-specific recovery has been reported by Shaughnessy and colleagues (1996). Frontal cortical ChAT activity was still decreased 12 weeks after lesioning of the nbm, whereas ChAT activity had recovered to normal levels in the parietal cortex. Unlike Wenk and Olton (1984), and Shaughnessy et al. (1996), Thal, Dokla, and Armstrong (1988), and Winkler and colleagues (1998) did not find any indication for recovery of cortical ChAT activity up to 3 months after bilateral nbm lesions. These results may imply that other processes such as collateral sprouting may have taken place after unilateral lesions.

In summary, we found clear age-associated impairments in the performance of sensorimotor tasks and in the acquisition of the seven-choice spatial discrimination task. Lesioning of the nbm did not affect the performance of the rats in the battery of sensorimotor tasks. We found only a transient effect on the acquisition of the seven-choice task. All rats were able to acquire this task; however, nbm-lesioned rats made more errors before they reached the criterion of seven error-free trials in a series of nine trials. The effects of the lesion were similar in both age groups. There were no differences in cortical ChAT activity between the young and aged rats, and lesioning of the nbm reduced cortical ChAT activity to a similar extent in both age groups. Our expectation that aged rats would be more susceptible to nbm lesion-induced degeneration, and that consequently, aged rats would show more severe behavioral dysfunctions as a result of lesioning the nbm than young rats, was not confirmed.

Thus, aged, nbm-lesioned rats are not a more appropriate model of Alzheimer's dementia than young nbm-lesioned rats.

3.4

Behavioral consequences of lesioning the nucleus basalis magnocellularis (nbm) in rats: discussion and conclusions

Lesioning of the nucleus basalis magnocellularis (nbm), the rodent homologue of the nucleus basalis of Meynert (nbM) in primates, has been suggested as a model of Alzheimer's disease (Wenk, Cribbs & McCall, 1984; Kesner, Adelstein & Crutcher, 1987; Shaughnessy et al., 1994, 1996). The cell loss in the nbM of patients suffering from Alzheimer's disease leads to a profound reduction of cholinergic projections to the neocortex. This cholinergic dysfunction is considered one of the major causes of the cognitive impairments seen in patients suffering from Alzheimer's disease (Bierer et al., 1995).

The animal model of the nbm-lesioned rat should mimic the reduction in cortical cholinergic activity and the concomitant impairment of cognitive functions. We designed this study to assess the effects of bilateral nbm lesions in rats, induced by injections of ibotenic acid, on cortical choline acetyltransferase activity and on performance in spatial orientation tasks, namely the holeboard, and the seven-choice task in an eight-arm radial alley maze. Both tasks have previously been found to be sensitive to the effects of normal aging (e.g. van der Staay, van Nies & Raaijmakers, 1990; Raaijmakers et al., 1990; see also Appendix 6.2). The holeboard allows the simultaneous assessment of both spatial working memory (WM) and reference memory (RM) (van der Staay, van Nies & Raaijmakers, 1990; Markel et al., 1995). The seven-choice task mainly relies on RM (Raaijmakers et al., 1990).

Table 1 summarizes the results of our experiments. The results of the first experiment support the notion, proposed by Wirsching and colleagues (1989) and by Givens and Olton (1994), that the nbm is involved in both spatial WM and RM. However, the seven-choice task, which taps predominantly RM, yielded inconclusive results. The acquisition of this task appeared to be slowed in young rats in the first experiment of Chapter 3.2, and in young and old rats in the experiment reported in Chapter 3.3. By contrast, no such effect was seen in young rats in the third experiment of Chapter 3.2. In the latter experiment, two different sets of lesion coordinates were used, whereas the injection volumes and the concentration of ibotenic acid were identical in all experiments. Both sets of lesion coordinates induced deficits in some experiments, but not in others (see Table 1).

Lesioning of the nbm did not affect performance once the rat had successfully acquired the task. This was true when we tested the retention of the seven-choice task in young adult and old rats, although the lesion retarded acquisition of this task in both age groups (Chapter 3.3). We also found that the lesion did not affect performance in the second experiment of Chapter 3.2., where rats had acquired the task to criterion before they received nbm lesions. Neither the retention test, nor the acquisition of a second problem, nor the re-acquisition of the originally acquired task, were affected by the nbm lesion.

Aged rats consistently show impaired learning and memory in spatial orientation and discrimination tasks. Moreover, Fischer, Gage, and Björklund (1989) found that both the size and the number of acetylcholinesterase-positive neurons in the forebrain nuclei were reduced in aged rats, when

compared with young conspecifics, whereas cortical ChAT activity was hardly affected by age. We hypothesized that aged rats would suffer more from ibotenic acid-induced lesions of the nbm than young rats, because we expected that the lesion would cause more damage in a system that already is compromised by age-associated degenerative changes.

Contrary to our expectations, however, we did not find a lesion by age interaction. There is experimental evidence that the sensitivity of neurons to the effects of neurotoxins is reduced in older animals (e.g. Luiten et al., 1995; Wenk et al., 1996) but there is also evidence for increasing sensitivity in older animals (Zawia, Arendash & Wecker; 1992). Wenk and colleagues (1996), using NMDA and AMPA to induce deficits in a delayed T-maze alternation task, found that aged rats were less affected by the nbm lesions than were young rats. In the aged rats the normally occurring age-related impairments and the toxin-induced damage combined produced behavioral deficits that were not different in their severity from the neurotoxin-induced behavioral deficits seen in young rats.

Table 1. Summary of the effects of bilateral lesions of the nucleus basalis magnocellularis (nbm) in rats on the performance in the spatial holeboard discrimination task and in the seven-choice task in an eight-arm radial alley maze. It is indicated whether the lesion was induced before or after the original acquisition (acquis.) of the learning task, which set of coordinates was used, how old the animals were, whether the lesion induced impairments, and in which Chapter (plus, where appropriate, the experiment number) the results are described in detail.

*: the anterior-posterior coordinates are with respect to bregma (Paxinos & Watson, 1986), the dorsoventral coordinates are with respect to the surface of the skull.

Task	The nbm was lesioned		Lesion coordinates*		Age of the animals		Lesion-induced impairments	Chapter/exp. no.
	before acquis.	after acquis.	AP: -0.5 L: \pm 2.3 DV: 7.6	AP: -0.8 L: \pm 2.7 DV: 8.0				
Holeboard: acquisition	before		●		young		yes	3.1
Seven-choice task: acquisition	before		●		young		yes	3.2 / 1
Seven-choice task: acquisition	before		●	●			no	3.2 / 3
Seven-choice task: acquisition	before			●	young	old	yes	3.3
Seven-choice task: retention	before			●	young	old	no	3.3
Seven-choice task: retention 1 st task		after	●		young		no	3.2 / 2
Seven-choice task: acquisition 2 nd task		after	●		young		no	3.2 / 2
Seven-choice task: re-acquisition 1 st task		after	●		young		no	3.2 / 2

If we consider only the experiments in which the nbm lesion preceded acquisition, then the results are inconclusive. In three of the four experiments with this experimental setup, nbm lesions impaired

(holeboard: Chapter 3.1; seven-choice task: Chapter 3.3) or prevented the acquisition of the task (seven-choice task: Chapter 3.2, first experiment), even after extended training.

The two sets of lesion coordinates we used are within the area identified by Wenk, Cribbs, and McCall (1984) to optimally induce selective reductions in cortical choline acetyltransferase (ChAT) activity. Comparison of the effects of lesioning of the nbm with ibotenic acid at the two sets of coordinates showed that the reduction in ChAT activity in cortical samples was greater when the coordinates AP: -0.8, L: \pm 2.7, and DV: 8.0 were used than when the coordinates AP: -0.5, L: \pm 2.3, and DV: 7.6 were used (third experiment of Chapter 3.2). The stronger impact of the lesion at the first set of coordinates was not accompanied by a greater effect on behavior, namely acquisition of the seven-choice task. In fact, in the experiment reported in Chapter 3.3, the lesioned rats made only slightly more errors to reach the criterion of seven error-free trials in a series of nine trials. The nbm lesions did not affect the number of trials made before the criterion was reached.

Although these results do not support the notion that the cortical cholinergic activity originating in the nucleus basalis is critically involved in memory, neither do they undermine it. There are a number of alternative explanations for these findings.

Dissociation between the decrease in cortical cholinergic markers and the severity of cognitive impairments

Dunnett, Everitt, and Robbins (1991) compared the effects of nbm lesions induced by different neurotoxins on performance of a broad range of learning and memory tasks. Their major finding was that the neurotoxins differ in their efficacy in destroying neurons of the nbm, with AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) being most effective, ibotenic acid being the least effective, and quisqualic acid being more effective than ibotenic acid, but less effective than AMPA. However, ibotenic acid-induced lesions of the nbm generally had the most profound effect on learning and memory in the Morris water escape task. None of the toxins selectively affected cholinergic neurons. Dunnett, Everitt, and Robbins (1991) therefore concluded that the effects seen on learning and memory are most likely due to non-specific destruction of non-cholinergic neurons. This view is also shared by Steckler and colleagues (1993), who concluded that the effects of ibotenic acid-induced lesioning of the nbm on subsequent cognitive performance are due to non-specific neuronal damage. Assessing the effects of quisqualic acid and ibotenic acid-induced lesions of the nbm in the Bättig radial arm maze, the Barnes circular platform, and in an operant autoshaping procedure, they found that the performance of the ibotenic acid but not of the quisqualic acid-lesioned rats was impaired in the Bättig maze and in the operant conditioning task. The performance of the lesioned rats in the Barnes maze was never different from that of the controls.

The Barnes maze task bears some resemblance to the seven-choice task we used in that one of the holes in the perimeter of the maze provides an escape route, whereas the other holes do not. The main difference between the Barnes maze and the seven-choice task is that abundant extra-maze cues are available in the circular maze. In contrast, the eight-arm radial alley maze we used provides almost exclusively intra-maze cues (see Chapter 3.2, Figs. 1 and 2). The Barnes maze thus is an allocentric task in which extra-maze cues guide a rat's orientation, whereas the 7-choice task might be solved as an egocentric task in which extra-maze cues are of minor significance.

Selectivity of ibotenic acid-induced lesions of the nbm for cholinergic cells

Ibotenic acid-induced lesions of the nbm are far from selective for cholinergic neurons (Roßner, Schliebs & Bigl, 1994). Ibotenic acid destroys cell bodies in the vicinity of the injection site, while

leaving fibers passing through this area intact. Therefore it is to be expected that injection of ibotenic acid into a region rich with cholinergic cells produces predominantly cholinergic dysfunctions. Nevertheless, depending on the precise site and size of the lesion, non-cholinergic neurons will also be damaged (e.g. Ofri et al., 1992; Steckler et al., 1993; Roßner, Schliebs & Bigl, 1994).

Animal models based on excitatory lesions of basal forebrain nuclei, such as the nbm, have severely been criticized because of the weak correlations or even lack of correlation between behavioral deficits and the magnitude of the decrease in cortical cholinergic markers (e.g. Dunnett, Everitt & Robbins, 1991; Torres et al., 1994). However, Alzheimer's disease is characterized by the degeneration of multiple transmitter systems (e.g. Olton & Wenk, 1987; Arai et al., 1992; Sparks et al. 1992). Focusing exclusively on the cholinergic system, or even more strictly, on the cholinergic projections from the nbm to the cortex, might reduce the face validity of animal models. Therefore, effects on neurotransmitters other than cholinergic one might be regarded as a potentially significant aspect of the face validity of the nbm-lesioned rat for Alzheimer's disease.

The cortical mantle in the rat receives massive input from the nucleus basalis, of which 80 to 90% appears to be cholinergic (Rye et al., 1984; Smith, 1988). Ibotenic acid and other non-selective toxins at best appear to destroy about 50% of the cholinergic projections. An approach to induce more selective damage to cholinergic neurons is thus needed in order to be able to evaluate the role of the cholinergic projections in lesion-induced cognitive impairments.

Recently, new techniques have been developed for inducing more selective lesions in the cholinergic system than the widely used neurotoxic glutamate analogues, such as kainic, ibotenic, quinolinic, or quisqualic acid (Smith, 1988; Wenk, 1996). Cholinergic neurons can be selectively damaged by injecting the monoclonal antibody against the p75 nerve growth factor (NGF) receptor, 192 IgG, coupled to the protein saporin, which inactivates ribosomes (Berger-Sweeney et al., 1994). The low affinity p75 NGF receptor is located on nearly all cholinergic neurons of the medial septal area (msa), and on the majority of cholinergic neurons in the nbm. Neurotransmitter systems other than the cholinergic ones appear to be unaffected by the immunotoxin 192 IgG-saporin (Torres et al., 1994). Earlier, Kudo and colleagues (1989) described a technique to selectively destroy cholinergic cells. They injected an NGF-diphtheria toxin conjugate into the cerebral cortex of rats and observed a decrease in ChAT-like immunoreactive neurons ipsilateral to the site of injection in the nbm. This technique, however, has not gained as much acceptance as the technique with 192 IgG saporin as immunotoxin. Infusion of the immunotoxin 192 IgG saporin has also been found to induce cholinergic deafferentiation in the basal forebrain nuclei (Holley et al., 1994). By using selective immunotoxins, it should be possible to study the role of the cholinergic projections originating in the nbm in cognitive processes more specifically.

For example, Berger-Sweeney and colleagues (1994) injected the immunotoxin 192 IgG saporin into the msa, the nbm, or the ventricles to produce cholinergic lesions, and then tested the rats in the Morris water escape task. All lesions impaired the spatial discrimination performance. The nbm lesion had no effect on the cued, i.e. non-spatial, version of the task. A weak correlation was found between the cholinergic fiber loss in the cortex and the severity of the immunotoxin-induced deficits in the spatial version of the Morris task.

In contrast, using the same neurotoxin to lesion the msa, nbm, or both, Dornan and co-workers (1996) did not see any effect of the lesions on the performance in the Morris water escape task, and only a slight effect on the performance in an eight-arm radial maze, in which a subset of arms was baited with food reward. Only WM performance appeared to be affected, whereas RM performance was not

different from that shown by the control animals. Dornan and colleagues (1996) concluded that the lesion-induced decrease in cholinergic neurotransmission in the basal forebrain is not sufficient to induce impairments in spatial orientation learning.

Sensitivity and selectivity of the behavioral tasks used to investigate cognitive processes critically depending on cortical cholinergic regulation.

It can be argued that the behaviors we assessed are not sensitive enough to detect the effects of cortical cholinergic depletion induced by lesioning of the nbm. Recently, the hypothesis has been forwarded that cholinergic systems predominantly have a role in attention processes (Dunnett, Everitt & Robbins, 1991; Connor, et al, 1993; Blokland, 1996; Voytko, 1996; Turchi & Sarter, 1997). Neither the holeboard task nor the seven-choice task is critically dependent on attention, although the processing of spatial extra- and/or intra-maze cues is relevant in these tasks. It is conceivable that the task(s) used must exceed a certain degree of complexity to become sensitive to cognition-disrupting effects of nbm lesions. In this respect, the seven-choice task appears to possess 'borderline sensitivity', i.e. its sensitivity seems to be insufficient to reliably detect nbm lesion-induced deficits.

The outcomes of experiments assessing the effects of nbm lesion-induced impairments in learning and memory are very heterogeneous. Neither the task demands, nor the lesions, can be directly compared between studies. Ibotenic acid lesions were found to impair spatial WM and RM, measured, for example, in the T-maze, the Morris water escape task, and the radial maze. However, clear deficits were reliably detected only after injection of volumes larger than the volume used in the present study, after multiple injections of the neurotoxic compound, or when the nbm lesions were combined with lesions of additional forebrain areas. Small lesions in this respect might be more selective than large ones. However, with small lesions, the magnitude of the damage might be insufficient to induce effects on behavior. Larger lesions, or series of lesions, which aim at the nbm in its entire extension, might damage too many surrounding structures so that the resulting behavioral deficits might be unspecific, or specific for the behavioral impairments induced by damage in structures adjacent to the nbm (Meyer & Coover, 1996). In this case the specific effects of lesioning the nbm cannot be distinguished from those caused by damage to surrounding structures. Dekker and colleagues (1991) suggest that the size of the lesion might provide the basis for the relation that is sometimes found between cortical ChAT depletion and the severity of neurological or cognitive deficits seen after ibotenic acid-induced nbm lesions.

Present status of ibotenic acid-induced lesions of the nbm

Measuring the effects of unilateral ibotenic acid-induced lesions of the nbm in rats, Roßner, Schliebs, and Bigl (1994) found a pattern of upregulation or down-regulation of cortical glutamate and GABA receptors which was highly similar to that seen postmortem in the cortices of patients who had suffered from Alzheimer's disease. They suggested that these changes mirror the activity of compensatory mechanisms triggered predominantly by cholinergic degenerative processes. The resemblance between the consequences of lesions of the nbm in rats and the consequences of cell loss in the nbM of Alzheimer's patients is considered to support the use of rats with nbm lesions as an animal model to assess the effects of putative therapeutics for Alzheimer's disease. Ofri and colleagues (1992) drew a similar conclusion, based on the similarities seen between changes in cortical opioid receptor binding in rats with ibotenic acid-induced lesions of the nbm and those seen postmortem in the brains of Alzheimer's patients.

Thus, although ibotenic acid lesions are less effective in reducing cortical cholinergic markers than, for example, quisqualic acid lesions, there appears to be support for ibotenic acid-induced lesions in the basal forebrain of rats as model for (part of the symptomatology of) Alzheimer's disease.

The rat model of nbm lesions, induced by ibotenic acid, is still frequently used to assess the effects of putative therapeutics for the treatment of Alzheimer's dementia (e.g. Kinoshita et al., 1992; O'Connell, Earley & Leonard, 1994; Hodges et al., 1995; Itoh et al., 1997). However, many of the points discussed above provide arguments against the use of this model. More sophisticated and selective techniques to model the cholinergic impairments seen in patients suffering from Alzheimer's disease are now available, and are to be preferred. A point in favor of the use of ibotenic acid to produce the lesions is the huge body of literature available in which this technique has been applied, and the reproducibility of the cognitive impairments induced, provided the lesions are big enough.

An acceptable model should mimic the selective loss of cholinergic neurons in the nbm and the behavioral deficits, predominantly memory deficits (Dunnett, Everitt & Robbins, 1991), which are symptomatic for Alzheimer's disease (American Psychiatric Association, DSM IV, 1994). Unfortunately, either the neurotoxins used appear to have limited selectivity with respect to the cholinergic projections, or they are highly selective, but do not reproducibly cause memory deficits. Therefore, the question about the degree of correspondence between the model and the disease which it is supposed to mimic finally depends on the purpose of the investigations (Maurer & Séguinot, 1995). Non-selective neurotoxins, such as ibotenic acid, quisqualic acid, AMPA, or NMDA, might provide the tools of choice to induce nbm lesions, if the aim of the study is to test the effects of putative Alzheimer's therapeutics on cognitive (dys)functions. To further elucidate the specific role of the cholinergic projections originating in the basal forebrain, selective immunotoxins such as 192 IgG saporin are the tools of choice (Fibiger, 1991; Torres et al., 1994).